

# Significance of the LL-37 Peptide Delivered from Human Cathelicidin in the Pathogenesis, Treatment, and Diagnosis of Sepsis

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## Abstract

Antimicrobial peptides, which function as the first line of host immune defense, have recently been identified as important immunomodulators of inflammation, and are involved as regulatory molecules in infections, including sepsis. Treatment of sepsis is very complex and remains largely challenging and sometimes ineffective. This creates a need to develop new therapeutic strategies focusing not only on the elimination of sepsis-causing microorganisms, which can be achieved with antibiotics, but also on the control of the immune system and its overactive response resulting in increased vascular endothelial permeability. One approach to develop new treatments for patients with sepsis is to better understand the pleiotropic function of the human LL-37 peptide that originates from the human cathelicidin antibacterial protein (h-CAP18). An increasing number of studies indicate high dynamics of changes in LL-37 concentration in the blood during sepsis. Additionally, in animal models, administration of exogenous LL-37 peptide to mice with experimentally induced sepsis increases their survival. It can therefore be assumed that knowledge of the molecular mechanism of cathelicidin LL-37 action, as well as the synthesis of its stable analogs, will result in progress in the diagnosis and therapy of sepsis.

## Keywords

Sepsis · Cathelicidins · LL-37 peptide · Bacteria

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## Abbreviations

ADP, adenosine 5'-diphosphate; ALX, lipoxin A<sub>4</sub> receptor; AMC, amoxicillin with clavulanic acid; AMPs, antimicrobial peptides; APACHE II, acute physiology and chronic health evaluation II; ATP, adenosynotriphosforan; BAEC, bovine aortic endothelial cells; BPI, bactericidal/permeability-increasing protein; cAMP, cyclic adenosine monophosphate; CAMP, cathelicidin antimicrobial peptide; CCL2, C-C motif chemokine ligand 2; CLP, cecal ligation and puncture; CRP, C-reactive protein; DAMP, damage-associated molecular patterns; DIC, disseminated intravascular coagulation; ELISA, enzyme-linked immunosorbent assay; ERK, extracellular signal-regulated kinases; FPRL, formyl peptide like receptor; FPR2, Formyl Peptide Receptor 2; FPR2/ALX, Formyl peptide receptor 2/lipoxin A4 receptor; HBP, heparin-binding protein; h-CAP18, human cathelicidin antibacterial protein; HMVEC, human microvascular blood vessel endothelial cells; hUC-MSC, human umbilical cord mesenchymal stem cells; HUVEC, human umbilical vein endothelial cell line; ICU, intensive care unit;

IFN, interferon; IL, interleukin; LAC, lactate concentration in arterial blood; LPS, lipopolysaccharide; LTA, lipoteichoic acid; MBC, minimum bactericidal concentration; MCR, mobilized colistin resistance; MIC, minimum inhibitory concentration; MPV, mean platelet volume; NaCl, sodium chloride; NET, neutrophil extracellular traps; NF-κB, nuclear factor κ-light-chain-enhancer of activated B cells; NLRP3, nod-like receptor family pyrin domain containing 3; P2X7, purinergic channel receptor; PAMP, pathogen-associated molecular patterns; PBMCs, peripheral blood mononuclear cells; PCT, prolactin; PLT, platelets; PRRs, pattern recognition receptors; SLE, systemic lupus erythematosus; TLRs, toll-like receptors; TNF, tumor necrosis factor; VE, Vascular Endothelial; VEGF, vascular endothelial growth factor; VEGFR2, Vascular Endothelial Growth Factor Receptor 2; WBC, white blood cell count.

## 1. Introduction

Sepsis is defined as a generalized, abnormal response of the body to an ongoing bacterial, viral, fungal, or parasitic infection, accompanied by a dysregulated inflammatory/immune response, resulting in excessive production of cytokines leading to multiple organ dysfunction. A critical element of the pathogenesis of sepsis is inflammation of the vascular endothelium, increasing its permeability and disturbance of the homeostasis of the volume of the vascular system. This leads

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to tissue hypoperfusion, hypoxia, and damage. An important feature of the inflammatory process developing in sepsis is the change in antimicrobial peptide (AMP) expression. Physiologically, these are substances, responsible, among others, for maintaining the microbiome balance of the skin barrier and mucous membranes, as well as eliminating microorganisms that have overcome these barriers. AMPs, also described as host defense peptides (Nagaoka et al. 2020), represent an interesting group of compounds as potential therapeutic measures to combat drug-resistant microorganisms (Makowska et al. 2019). Upon insertion into bacterial membranes, they lead to disturbances and loss of membrane barrier functions and polarization (Guilhelmelli et al. 2013). They are active against Gram-positive and Gram-negative bacteria, viruses, fungi (Nagaoka et al. 2020), and protozoa (Pahar et al. 2020). In addition to their effective antimicrobial activity, they can regulate the host immune response – they constitute a link between the innate and adaptive response (Nagaoka et al. 2020); some of them inhibit the pro-inflammatory response by sequestering bacterial cell wall products (lipopolysaccharide [LPS]-binding) from their Toll-like receptors (TLRs), the growth of bacterial biofilm, and support the wound healing processes (Makowska et al. 2019). AMPs are divided into three main families:  $\beta$ -defensins, S100 proteins, and cathelicidins (Nagaoka et al. 2020). Here we characterize the human cathelicidin antibacterial protein (h-CAP18), LL-37 peptide and present its involvement and application potential in the pathogenesis and treatment of sepsis.

## 2. General Characteristics of the LL-37 Peptide

### 2.1. Biogenesis

LL-37 peptide expression is found in different human cells and tissues (Yang et al. 2020). Its presence has also been reported in body fluids (Chen et al. 2004; Bucki et al. 2007; Chinipardaz et al. 2022). In most epithelial cells, its expression is constitutive and regulated by signaling pathways involving cAMP synthesis (Bandurska et al. 2015). Cathelicidin is also expressed in cells of the innate immune system: neutrophils, natural killer cells, and mast cells, which store the precursor in their granules and constitutively express the cathelicidin protein (Vandamme et al. 2012). During infection or tissue damage, the TLRs are activated by microorganism products in a specific cytokine environment, leading to cell activation and degranulation (Bandurska et al. 2015). Additionally, dendritic cells, monocytes, macrophages, lymphocytes, mesenchymal stem cells, and bone marrow stroma have also been shown to express the LL-37 peptide (Vandamme et al. 2012). The expression of the LL-37 peptide can also be induced by endogenous and exogenous stimuli (Yang et al. 2020), with 1,25-dihydroxycholecalciferol underlined as one of the most important factors. Overall, the

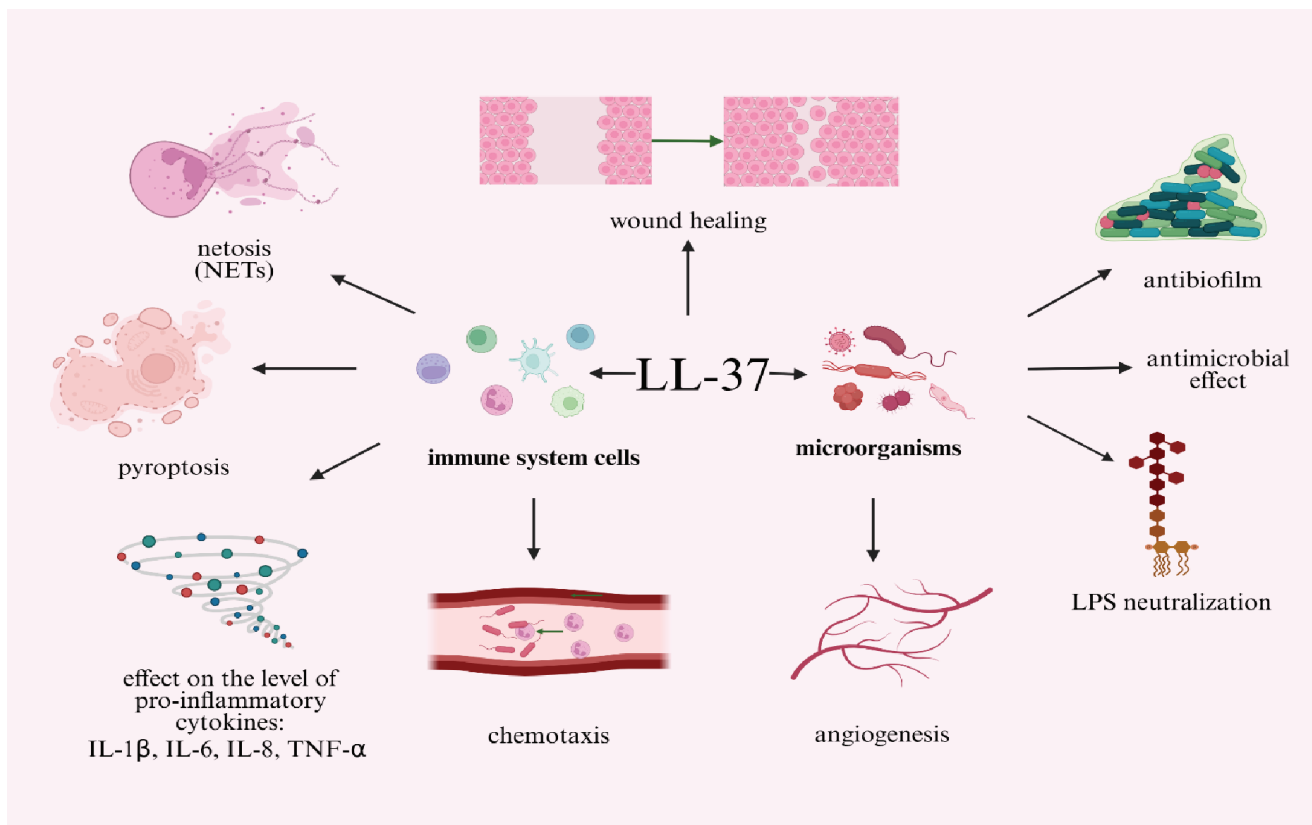
expression level of LL-37 depends on age and sexual maturation (Vandamme et al. 2012). LL-37 is a C-terminal host defense peptide produced by the proteolytic release of 37 amino acids sequences from h-CAP18 (Yang et al. 2000). The peptide is encoded by the *CAMP* gene and synthesized as a pro-form of h-CAP18. Pro-peptide is released, then cleaved extracellularly by serine protease 3 or kallikrein 5, and the biologically active peptide LL-37 is released (Nilsson 2020).

### 2.2. Functions of the LL-37 peptide with particular emphasis on immunomodulatory functions and role in the pathogenesis of sepsis

LL-37 sequence (LLGDFFRKSKEKIGKEFKRIVQRIKDFLRN LVPRTES) is reached in positively charged amino acids (Qin et al. 2019). Its functions are defined by cationic charge, the presence of amphipathic domains, and  $\alpha$ -helical structure (Wang 2008). The LL-37 peptide occurs in various structural forms and its biological activity depends on them (Engelberg and Landau 2020; Zeth and Sancho-Vaello 2021; Pavelka et al. 2024). The conformational variability and different oligomeric states of LL-37 enable it to adapt to different targets and influence its pleiotropic functions (Zeth and Sancho-Vaello 2021). As a result of the action of bacterial proteases, that is, proteinase K or peptidase, the LL-37 peptide can be cleaved into the hLL-37 peptide formed from residues 17–19, which directs interactions with bacterial cells by forming superhelical fibrils (Engelberg and Landau 2020). The main sites of interaction with lipids and LPS were defined as conserved phenylalanine and arginine residues of the LL-37 peptide, however, interactions with proteins or DNA are still unexplored, which is another direction for future research (Zeth and Sancho-Vaello 2021).

LL-37 has many important biological functions, as shown in Figure 1 (Alexandre-Ramos et al. 2018; Nagaoka et al. 2020; Yang et al. 2020). The biological activity of the LL-37 peptide depends on the physiological environment. Some experimental studies have described that the action of LL-37 is dependent on sodium chloride (NaCl), which decreases LL-37 antimicrobial properties, but in such environments LL-37 can still perform immunomodulatory functions (Mookherjee et al. 2006). Magnesium and calcium ions have also been described as inhibitors affecting the function of LL-37 (Turner et al. 1998; Bowdish et al. 2005). Design of new antibacterial substances may be based on molecular scaffolds of the LL-37 peptide with specific biological functions. However, stability in physiological solutions and bioavailability should be taken into account.

The most important basis for the pathogenesis of sepsis is an imbalance of inflammation in the body (Huang et al. 2019) that includes leukocytes (neutrophils, macrophages, natural killer cells), endothelial cells, cytokines, complement products, and the coagulation system (van der Poll et al. 2021). Activation of the immune response in sepsis



**Fig 1.** The pleiotropic function of human cathelicidin LL-37. In the extracellular environment, the LL-37 peptide has various functions resulting from its ability to activate certain plasma membrane receptors and/or to insert into the plasma membrane, thereby disrupting the integrity of the plasma membrane. LL-37 may also interact with substances that build microbial cells and components of the biofilm matrix. IL, interleukin; LPS, lipopolysaccharide; NETs, neutrophil extracellular traps; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ .

involves pattern recognition receptors (PRRs) (Jedynak et al. 2012), which are expressed in cells of the immune system and parenchymal cells (van der Poll et al. 2021). TLRs are the best-identified group of PRRs (Jedynak et al. 2012); thanks to these receptors, the body can recognize exogenous molecular patterns associated with pathogens (pathogen-associated molecular patterns [PAMP]) and endogenous molecular patterns associated with endogenous damage (damage-associated molecular patterns [DAMP]). Host–pathogen interaction leads to an immune response dedicated to fighting the microorganism and returning to homeostasis by mechanisms involving the subsequent activating of inflammatory and anti-inflammatory pathways at the site of infection. It is recognized that the nature/strength of the immune response might result in “good, bad, or ugly consequences” for the host. The good ones are associated with the effective elimination of pathogens by a well-regulated immune response. The ability of pathogens to bypass immune defense mechanisms causes PRRs to be overactivated, leading to excessive host response (“bad” consequences), inflammation and sepsis (Wiersinga and van der Poll 2022). In some instances, the

inability to control the infection and inadequate inflammation contribute to the development of septic cardiomyopathy (Xue et al. 2022), acute respiratory distress syndrome (Crimi and Slutsky 2004), and disseminated intravascular coagulation (DIC) (Popescu et al. 2022).

Studies performed by Nagaoka et al. (2020) using a mouse model of sepsis prove that the LL-37 peptide administered intravenously supports the survival of cecal ligation and puncture (CLP) mice by inducing the release of neutrophil extracellular traps (NETs) from neutrophils, facilitating the control of infections, as well as by stimulating neutrophils to release ectosomes with antibacterial properties. Moreover, the beneficial effects of LL-37 administration via the promotion of neutrophil-derived ectosomes were also confirmed by Kumagai et al. (2020). The LL-37 peptide inhibits apoptosis of neutrophils as well as induces secondary neutrophil necrosis (Alalwani et al. 2010). The ability to regulate NET production is a key element in the treatment of sepsis (Ou et al. 2022) because excessive NET formation causes damage to healthy host cells, resulting in inflammation and tissue damage (Yang et al. 2020). Studies conducted by Alalwani et al. (2010) have proved that LL-37 modulates the response of

neutrophils to LPS, reducing the release of pro-inflammatory cytokines interleukin (IL)-1 $\beta$ , IL-6, IL-8, and tumor necrosis factor (TNF)- $\alpha$  from activated neutrophils and reactive oxygen species production. On the contrary, it stimulates phagocytosis. Additionally, LL-37 inhibits neutrophil infiltration and migration via focal adhesion kinase and extracellular signal-regulated kinases (ERK) and the P38 pathway (Yang et al. 2020).

Pyroptosis is a phenomenon requiring two types of PAMP and DAMP stimuli and occurs mainly in macrophages and dendritic cells. It is caspase-1-dependent cell death, accompanied by the release of pro-inflammatory cytokines, cell lysis, and the release of cytosol from the cell, which exacerbates the inflammatory reaction (Hu and Nagaoka 2016). LL-37 peptide inhibits LPS/Adenosinetriphosphor (ATP)-induced macrophage pyroptosis *in vitro* (Hu et al. 2014). Studies performed using a mouse model of sepsis confirm that LL-37 inhibits macrophage pyroptosis and the release of inflammatory cytokines IL-6, TNF- $\alpha$ , and IL-1 $\beta$ , improving the survival of mice with CLP sepsis (Hu et al. 2016). *In vitro*, it inhibits the production of TNF- $\alpha$  by human monocytes and macrophages stimulated with LPS or lipoteichoic acid (LTA) (Sun et al. 2014). LL-37 also takes part in the differentiation of monocytes. Additionally, LL-37 in macrophages and dendritic cells interrupts the functions of the TLR4 complex, leading to a decrease in the production of inflammatory cytokines in the presence of LPS (Chinipardaz et al. 2022). In LPS-stimulated murine macrophages, it reduces TNF- $\alpha$  production by inhibiting the activation of p38 and ERK (Castillo et al. 2022). LL-37 during infection or inflammation supports the pro-inflammatory and anti-infective functions of monocytes/macrophages while controlling the level of inflammation and maintaining the body's immune balance (Yang et al. 2020). In alveolar epithelial cells LL-37 modulates the expression of nod-like receptor family pyrin domain containing 3 (NLRP3), caspase 1, and gasdermin D, protecting mice against LPS-induced septic lung injury (Wang et al. 2024). Animal model studies have shown beneficial activity of the LL-37 peptide in sepsis, but it should be noted that differences in the immune systems of mice and humans should be considered when the translational conclusions are drawn about the potential benefit of administration of LL-37 in humans. Further studies are needed to assess whether the results obtained in animal models will translate to activity in humans (Coorens et al. 2017).

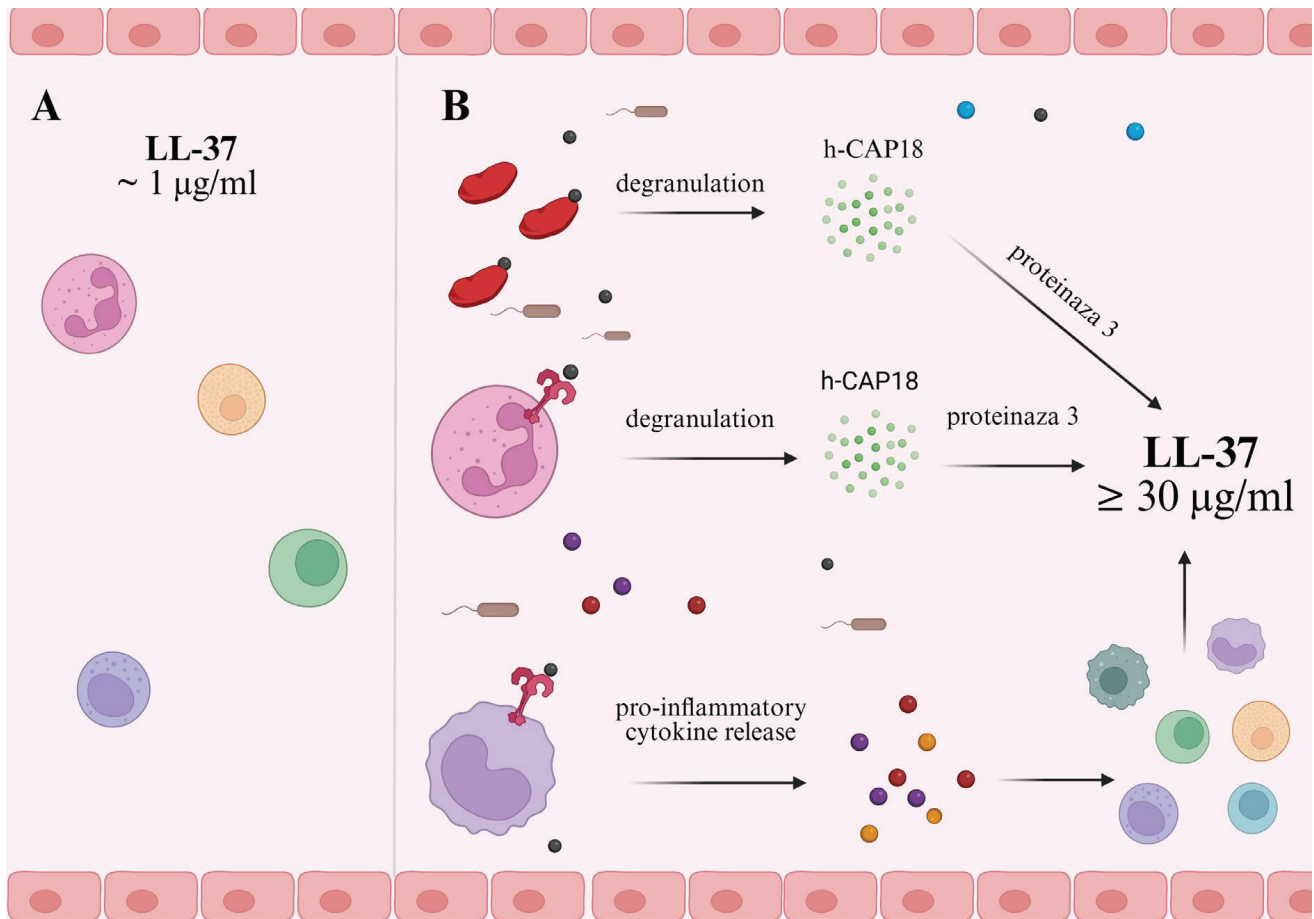
The LL-37 peptide affects the viability of peripheral blood mononuclear cells (PBMCs); it does not affect the activation of T lymphocytes, which was detected by assessing the expression of the CD69 membrane receptor in the resting state and conditions of inflammation. Additionally, it can induce the proliferation of resting phytohemagglutinin-activated T cells, increase the production of Tregs (which play a key role in maintaining immune homeostasis), and

reduce the expression of pro-inflammatory transforming growth factor  $\beta$  as well as TNF- $\alpha$  and interferon (IFN)- $\gamma$ , which indicates that the peptide regulates the pro-inflammatory response (Alexandre-Ramos et al. 2018). IL-1 $\beta$  synergistically supported by LL-37 peptide increases the secretion of chemokine (CC motif) ligand 2 (CCL2), IL-6, and IL-10 in PBMCs (Bandurska et al. 2015).

### 3. Changes in the Concentration and Expression of the LL-37 Peptide in Infections, with Particular Emphasis on Sepsis. LL-37 as a Diagnostic Marker for Monitoring Disease Progress and Treatment Effects

In healthy people, the plasma concentration of LL-37 is ~1  $\mu\text{g/mL}$  and during inflammation and activation of the immune system, its level significantly increases (Nagaoka et al. 2006), even to 30  $\mu\text{g/mL}$  (Perez-Rodriguez et al. 2022). Controlling inflammatory mediators has become one of the main goals in drug development for the treatment of sepsis (Huang et al. 2019). Figure 2 shows a diagram illustrating the expression of LL-37 induced by an inflammatory factor during sepsis.

Septic patient status and risk of increased mortality correlate with plasma AMP levels (Berkestedt et al. 2010). Miao et al. (2022) determined the level of LL-37 using enzyme-linked immunosorbent assay (ELISA) in cord blood collected from premature infants during delivery. On the third day after birth, they determined parameters such as white blood cell count (WBC), platelets (PLT), mean platelet volume (MPV), and C-reactive protein (CRP) concentration. Compared with the control group, an increase in the concentration of LL-37 peptide, CRP, WBC, and MPV was observed in premature infants with sepsis. The PLT level was significantly lower than in the control group. LL-37 peptide expression correlated positively with the MPV level and negatively with the PLT level. These observations suggest the possibility of monitoring early neonatal sepsis by assessing these parameters. Based on this, it can be concluded that an elevated level of LL-37 may indicate the risk of sepsis in the initial phase of its development (Miao et al. 2022). Early diagnosis of patients with sepsis may also be based on simultaneous measurement of LL-37, procalcitonin (PCT) and CRP levels. The study conducted by Liu et al. (2019) on 40 patients diagnosed with sepsis and 20 healthy volunteers consisted of assessing: the expression of the LL-37 peptide and the level of CRP and PCT. The level of the tested markers in peripheral blood was higher than in the control group. A positive correlation was also observed between PCT, CRP levels, and LL-37 peptide expression (Liu et al. 2019). Guo et al. (2018) assessed the level of LL-37 in the peripheral blood of

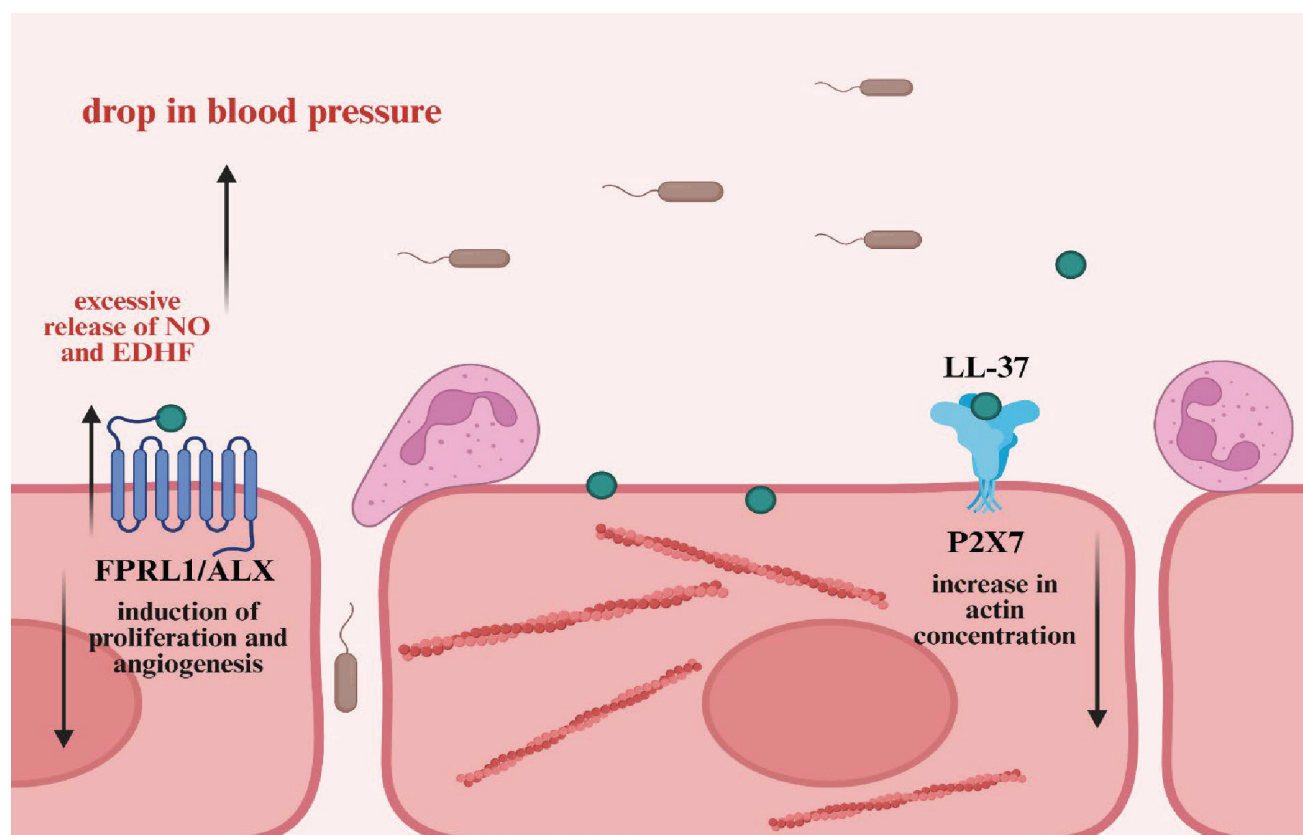


**Fig 2.** Scheme of LL-37 peptide expression by immune cells during homeostatic balance (A) and during sepsis (B).

patients over 65 years of age, simultaneously monitoring the critical condition indicators (CRP, PCT), lactate (Lac), concentration in arterial blood, and the extent of organ failure using the sequential organ failure assessment (SOFA) score index and the results of acute and chronic health assessment II (APACHE II) on days 1, 3, and 7 from the decision to hospitalize. People taking part in the study were divided into the following groups: I. Healthy people ( $n = 32$ ); II. Elderly people with community-acquired pneumonia ( $n = 31$ ); III. People with sepsis ( $n = 67$ ); and IV. People with septic shock ( $n = 46$ ). PCT, CRP, Lac, APACHE II, and SOFA were elevated in hospitalized patients compared with healthy subjects, and these parameters increased as the condition of patients worsened. Interestingly, the level of LL-37 was higher in groups II–IV compared with group I, but with prolonged hospitalization in patients with sepsis and septic shock, the peptide level decreased compared with people with pneumonia. The level of LL-37 correlated negatively with the APACHE II and SOFA index, and a decrease in the level of LL-37 was associated with higher mortality (Guo et al. 2018). In another study using the ELISA to test samples from 31 adult patients of an

intensive care unit (ICU) with severe sepsis or septic shock, the level of the following factors was determined: bactericidal/permeability-increasing protein (BPI), heparin-binding protein (HBP), defensins, lactoferrin, LL-37, IL-6, IL-10, myeloperoxidase, CRP, and WBC. Compared with the control group, the level of the tested proteins was increased in patients with sepsis, however, except for the LL-37 peptide. BPI level was associated with increased mortality, while HBP defensins and lactoferrin did not correlate with mortality. On day 4, there was a decrease in BPI and HBP levels, and the levels of defensin and lactoferrin did not change compared with the day of entry into the study. Contradictory to previously described studies, the mean level of LL-37 peptide was similar in the control and sepsis patients; the peptide level did not correlate with mortality, and its concentration was not significantly different over time (Berkestet et al. 2010). In the following reports, a group of 183 patients of the Hospital – das Clinicas University of Sao Paulo (23 healthy people, 69 patients admitted to the ICU for non-infectious reasons, 28 with severe sepsis, 46 admitted due to septic shock, and 17 in the convalescence phase) was included in the study to assess the level of LL-37





**Fig 3.** Ability of the peptide LL-37 to regulate the stiffness and cytoskeleton organization of the endothelial cell. EDHF, endothelium-derived hyperpolarizing factor; FPRL1/ALX, formyl peptide receptor-like 1/lipoxin  $A_4$  receptor; NO, nitric oxide; P2X7/purinergic channel receptor.

in plasma by ELISA. Information about the health status of patients and blood samples were collected upon hospital admission or when severe sepsis or septic shock was diagnosed by medical personnel. Each patient enrolled in the convalescence phase came from the septic shock group; according to the assessment of the medical staff, the patient showed clinical and laboratory improvement in the infection. LL-37 levels are reduced during septic shock and double during the convalescent phase compared with healthy volunteers, which may indicate its important role in promoting tissue repair (LL-37 acts as potential agonist of growth factor receptors). The reduction in levels may mute further inflammatory responses, which is a potential defense mechanism (Barbeiro et al. 2013). Based on the above results, the level of LL-37 is influenced by many variables, such as the time at which the peptide level was measured, the stage of the sepsis, and age. LL-37 is a potential candidate for monitoring the development of sepsis and treatment effects in combination with the assessment of other parameters. However, it should be strongly emphasized that further clinical studies are necessary to assess the potential of LL-37 as a diagnostic marker in the management of sepsis. When assessing

LL-37 levels in plasma, caution should be taken because its changes in blood concentration are very rapid and dynamic. Granulocyte degranulation may result in higher concentrations of this peptide if blood samples are not held appropriately after collection.

#### 4. The Role of Vitamin D in Sepsis

Many reports indicate that the expression of the LL-37 peptide is to a large extent regulated by the active form of vitamin D (1,25(OH) $_2$ D) (Majewski et al. 2018). Immunological, endocrine, and endothelial functions are linked to vitamin D-mediated pathways (Delrue et al. 2023). Deficiency of this vitamin often occurs in patients in ICUs (about 70%), predisposing them to the development of severe infections and sepsis (Cutuli et al. 2024). In the liver, the metabolism of vitamin D begins. The enzyme 25-hydroxylase from the cytochrome P450 family (mainly CYP2R1) located on the endoplasmic reticulum converts vitamin D into 25-hydroxyvitamin D [25(OH)D, calcidiol]. Then the enzyme 1 $\alpha$ -hydroxylase (cytochrome p450, CYP27B1-mitochondria) of renal tubular cells into the most biologically

active 1,25-dihydroxyvitamin D [1,25(OH)<sub>2</sub>D, calcitriol]. Immune system cells (lymphocytes, macrophages, dendritic cells) express the vitamin D receptor on their surface (Jeng et al. 2009). 25-Hydroxyvitamin D is a clinical marker used to assess the level of vitamin D in the blood (Cutuli et al. 2024). Vitamin D and its metabolites stabilize the endothelium and prevent vascular leakage (Guan et al. 2023) ensuring hemodynamic stability (Delrue et al. 2023). Critically ill patients suffer from vitamin D deficiency, which increases mortality; higher levels of 25-hydroxyvitamin may reduce the risk of sepsis (Guan et al. 2023). Jeng et al. (2009) determined the concentrations of 25(OH)D and vitamin D-binding protein in plasma and their relationship with systemic LL-37 concentrations in critically ill patients with and without sepsis, comparing the results with healthy people. Critically ill patients had significantly lower concentrations of 25(OH)D and LL-37 in plasma compared with healthy people (Jeng et al. 2009). Oral administration of vitamin D supports the bactericidal effect and synthesis of LL-37 in human cells. In the culture of human bronchial epithelial cells with the addition of 1,25(OH)<sub>2</sub>D<sub>3</sub>, an increase in the concentration of the LL-37 peptide and inhibition of the growth of *Bordetella* and *Pseudomonas* strains were observed (Shawky et al. 2022). Macrophages infected with *Mycobacterium tuberculosis* treated with 1,25(OH)<sub>2</sub>D<sub>3</sub> were characterized by increased expression of LL-37. Reduced cathelicidin secretion induced by vitamin D deficiency inhibited *Mycobacterium* killing. Vitamin D deficiency is associated with a high mortality rate in patients with sepsis and is also a risk factor for sepsis (Jeng et al. 2009). An increased level of LL-37 in the serum, a decrease in the concentration of PCT, and clinical improvement were obtained after administration of a large dose of enterally supplemented vitamin D<sub>3</sub> in combination with the standard treatment in patients with sepsis requiring mechanical ventilation (Ashoor et al. 2024). Research by Quraishi et al. (2015) also highlights the importance of vitamin D supplementation in patients with severe sepsis or septic shock. Administration of cholecalciferol supported the expression of endogenous LL-37 peptide and normalized the concentration of blood cytokines (Quraishi et al. 2015), reducing mortality among hospitalized critically ill patients especially when vitamin D<sub>3</sub> concentration is below 12 ng/mL (Amrein et al. 2014). Supplementation in patients with sepsis may support the treatment effect by engaging host cells to produce endogenous LL-37 peptide.

## 5. LL-37 as a Regulator of Hemostasis and Vascular Permeability

### 5.1. Influence on the coagulation system

Hemostatic disorders accompanying sepsis include changes in coagulation parameters, activation of the coagulation

system with a decrease in platelet count, and prolonged coagulation time, which may result in extensive microthrombosis and profuse bleeding, the so-called DIC (Zhu et al. 2023). PLT are responsible for maintaining adequate hemostasis and actively participate in inflammatory processes (Vardon-Bouines et al. 2019). During pathophysiological conditions, excessive activation of thrombocytes occurs, which leads to the formation of clots and intravascular coagulation. Thrombocytopenia is the second phenomenon related to ongoing inflammation, which involves a disturbed clot formation, a mechanism leading to blood loss (Salamah et al. 2018). LL-37 peptide can activate PLT as an agonist of FPR2/lipoxin A<sub>4</sub> receptor (ALX) receptor. *In vitro*, LL-37 concentration in the range of 10–50 μM stimulates PLT to form clots, thus modulating hemostasis. At concentration ≥100 μM, LL-37 causes platelet damage, leading to hemorrhagic disorders. Elevated LL-37 levels during inflammatory diseases can lead to platelet-related complications such as thrombocytopenia and inflammation. Blocking the FPR2 receptor prevents platelet activation by the LL-37 peptide (Salamah et al. 2018). In addition to its hemostatic function, the LL-37 peptide supports the antimicrobial properties of human PLT (Sánchez-Peña et al. 2023). Another study assessed the effect of the peptide on human platelet aggregation *in vitro* and thrombus formation *in vivo*. LL-37 inhibited human platelet aggregation induced by collagen, adenosine 5'-diphosphate (ADP), and U46619 in a dose-dependent manner. The stronger effect was observed after the addition of LL-37 at a dose of 1.2 μM. In a rat model of arteriovenous thrombosis, LL-37 had antithrombotic functions. Most specifically, LL-37 inhibited thrombosis formation as the mass of the clot *in vivo* was reduced upon administration of LL-37 at a dose of 15 mg/kg (Su et al. 2016).

### 5.2. Regulation of vascular permeability and pressure

Under physiological conditions, the vascular endothelium is responsible for maintaining proper hemostasis (Ince et al. 2016). Impaired capillary permeability occurring during sepsis leads to increased migration into tissue and adhesion of leukocytes to the endothelium, excessive release of nitric oxide (NO) and prostacyclin causing vasodilation (decrease in blood pressure), increased endothelial permeability, and loss of barrier function, resulting in extensive tissue edema and organ failure (Ince et al. 2016; Dolmatova et al. 2021). Understanding the mechanisms of increased endothelial permeability allows for more effective development of therapeutic strategies related to vascular barrier dysfunction that accompanies sepsis. LL-37 is capable of activating G protein-coupled receptors, such as formyl peptide-like receptor 1 (FPR1), tyrosine kinase receptors such as epidermal growth factor receptor, TLR, and P2X<sub>7</sub>R through which it affects the cell cytoskeleton functions of endothelial cells (Yanagisawa

et al. 2020) (Figure 3). The reorganization of the endothelial cell cytoskeleton is regulated by cell signaling and is associated with actin remodeling, causing changes in cell stiffness and permeability (Xiong and Hla 2014). The effect of the LL-37 peptide on the stiffness and permeability of endothelial cells was recently assessed. The research used the human umbilical vein endothelial cell line (HUVEC), bovine aortic endothelial cells (BAEC), human pulmonary microvascular endothelial cells, and mouse aorta. The study using atomic force microscopy showed an increase in the stiffness of the endothelial cell cytoskeleton observed during the addition of increasing concentrations of LL-37. The stiffening induced by LL-37 depends on the P2X7 receptor and the intracellular  $\text{Ca}^{2+}$  concentration, as evidenced by the fact that BAEC cells treated with a P2X7 receptor antagonist (KN-62) or calcium chelation showed lower LL-37-induced stiffness. HUVEC monolayer showed decreased permeability (as assessed using FITC-dextran) in the presence of LL-37 peptide, which was associated with increased F-actin concentration; in the mentioned study, no significant differences were observed in the organization of  $\beta$ -catenin or VE-cadherin. Restoration of barrier function is an important element in restoring hemostatic balance. Increased permeability can be regulated by LL-37 peptide, which at increasing concentration reduced permeability while increasing endothelial cell stiffness (Byfield et al. 2011). LL-37-mediated activation of FPRL1 in endothelial cells induces their proliferation and promotes angiogenesis, which enables the repair of endothelial cell disruption (Xhindoli et al. 2016). In addition to its direct angiogenic effects on endothelial cells, LL-37 attracts neutrophils and monocytes, which release angiogenic mediators in response to cellular activation (Koczulla et al. 2003). *In vitro*, immobilized LL-37 stimulated the proliferation of HUVEC endothelial cells by imitating the action of vascular endothelial growth factor (VEGF). This effect was, in part, mediated by activation of VEGFR2. This role may support the regeneration of a damaged endothelium (Szulcek et al. 2018). It is believed that in pathophysiological conditions, LL-37 can be used to control the disturbed process of vascular hemostasis by controlling cellular stiffness.

As a result of infection, the nuclear factor  $\kappa\text{B}$  (NF- $\kappa\text{B}$ ) pathway is activated, and pro-inflammatory cytokines TNF- $\alpha$  and IL-1 $\beta$  are released, which causes vascular leakage. Higher activity of this signaling pathway in patients with sepsis was associated with a higher risk of mortality (McMullan et al. 2024). LL-37 prevents LPS-induced translocation of NF- $\kappa\text{B}$  p50 and p65 subunits, which induce pro-inflammatory gene expression (Leite et al. 2023). During sepsis in the lungs and the liver, bacterial cell membrane components, such as LPS, induce apoptosis of endothelial cells, leading to circulatory disorders and organ dysfunction. LPS-induced apoptosis of human microvascular blood vessel endothelial cells

(HMVEC-LBIs) was suppressed in the presence of LL-37. The same effect was obtained in an animal model (Suzuki et al. 2011). Vasodilation accompanies systemic infection (septicemia), resulting in a drop in blood pressure, followed by hypoxia and organ damage. In human veins, via the ALX receptor also known as FPRL1, LL-37 induces endothelium-dependent relaxation, which involves the release of NO and endothelium-derived hyperpolarizing factor (Berkestedt et al. 2008). Promoting angiogenesis and vasodilation induced by overexpression of LL-37 peptide suggests a protective role against sepsis-induced organ damage including acute lung injury (Qin et al. 2019). Treatment involving ALX agonists may be an effective form of sepsis therapy because it will prevent vasodilation in patients with sepsis (Bucki et al. 2010). According to the above reports, the LL-37 peptide may find future application as a factor controlling vascular hemostasis.

## 6. LL-37 Receptor Activation and Role in Autoimmune Diseases

LL-37 is an important element of the immune response in autoimmune diseases such as systemic lupus erythematosus (SLE), psoriasis (Pahar et al. 2020). In patients with psoriasis, the peptide binds to the released DNA of damaged cells, creating a complex that activates the TLR9 receptor, which leads to the initiation of a pro-inflammatory response and the release of type I IFN (Scheenstra et al. 2020). A similar effect was observed in patients with SLE (Pahar et al. 2020). Through the P2X7 receptor on monocytes, it induces the release of IL-1 $\beta$  (Elssner et al. 2004) and IL-18 (Kahlenberg et al. 2013). A new direction of research may be to understand the molecular mechanisms of the LL-37 peptide on membrane receptors in sepsis models. This knowledge will enable control of the anti-inflammatory and pro-inflammatory response.

## 7. Etiological Factors of Sepsis and Activity of the LL-37 Peptide Against These Pathogens

### 7.1. Sepsis with bacterial etiology

As a result of an ongoing infection such as urinary tract infection, abdominal infection, pneumonia, or meningitis (Minasyan 2019), bacteria may enter the bloodstream, which carries the risk of developing sepsis (Holmes et al. 2021). Children, pregnant women, the elderly, people with weakened immune systems, and people with comorbidities are particularly at risk (Grondman et al. 2020). Important risk factors also include catheters, intravascular punctures, prostheses, medical devices, blood or fluid transfusions, enteral nutrition, and open wounds such as diabetic foot and burn wound



(Gauer et al. 2020). Translocation of microorganisms through damaged gastrointestinal mucosa may constitute a pathway for infection of the bloodstream (Vaishnavi 2013). Bacterial infections are the most common etiological factor of sepsis (Gauer et al. 2020). Over 60% of positive blood cultures are G-negative bacteria and over 40% are Gram-positive isolates (Dolin et al. 2019). Particularly dangerous multidrug-resistant pathogens are bacteria from the ESKAPE group (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp. (Marturano and Lowery 2019)). The main stimulus associated with pathogens contributing to the occurrence of sepsis is cell wall components LPS, teichoic acids, and peptidoglycan, which are promoters of the activation of the pro-inflammatory response (Cheung et al. 2021). As a result of antibiotic therapy, cell division, or apoptosis, bacteria are damaged, lysed, and endotoxin is released (Dickson and Lehmann 2019). LPS forms a complex with CD14 protein through the LPS-binding protein, then activates the NF- $\kappa$ B transcription factor with the participation of the transmembrane TLR4, leading to the activation of genes encoding pro-inflammatory cytokines IL-6, IL-1 $\beta$ , TNF- $\alpha$ , NO and others. Blood plasma components, such as lipoproteins, can inhibit the antimicrobial activity of the LL-37 peptide. Moreover, its endotoxin-neutralizing capabilities might be suppressed by exposure to inflammatory cell-released enzymes, namely, peptidyl arginine deiminases, resulting in citrullination of the peptide and abrogating its immunomodulatory functions (Koziel et al. 2014). Hence, there are few reports in the scientific literature on the antimicrobial activity of the LL-37 peptide in the treatment of sepsis in animal models, as this treatment usually requires intravenous administration of therapeutic agents. In one of the studies, LL-37 was reported to be as potent as imipenem in reducing the bacterial burden in *Escherichia coli*-induced septic rats and display comparable anti-endotoxin activities to polymyxin B (Cirioni et al. 2006). CAP18/LL-37 was also reported to be efficient against sepsis in neonatal rats (Fukumoto et al. 2005). In another report, Cirioni et al. (2008) demonstrated that coadministration of LL-37 and colony-stimulating factors is even more efficient against *P. aeruginosa*-induced sepsis than imipenem, presenting a novel therapeutic strategy to manage bacterial infections complicated by neutropenia. By destabilizing the bacterial cell membrane, cationic LL-37 allows greater penetration of the other antibiotic into bacterial cells (Ridyard and Overhage 2021), indicating that combined treatment with conventional antibiotics might be beneficial. For the *E. coli* strain carrying *mcr-1* gene, and producing an extended spectrum  $\beta$ -lactamase and carbapenemase, the minimum inhibitory concentration (MIC) of LL-37 ranged from 16 mg/L to 64 mg/L. LL-37/colistin combination showed synergistic activity against all tested strains (Morroni et al. 2021).

*In vitro*, the MIC for six clinical strains of *S. aureus* cultured from a wound with various resistance mechanisms and the reference strain for LL-37 was 3.125  $\mu$ M and the minimum bactericidal concentration (MBC) was 6.25  $\mu$ M. MIC values for amoxicillin with clavulanic acid (AMC) in combination with the LL-37 peptide (concentration 0.8  $\mu$ M) were lower for all seven strains compared with the previously used monotherapy. Amikacin in combination with LL-37 produced different effects depending on the resistance mechanism (no dose reduction against methicillin-resistant *S. aureus* strains). A synergistic effect was obtained after combining AMC with LL-37 (Leszczyńska et al. 2010). A better therapeutic effect was also obtained when the LL-37 peptide (MBC in monotherapy 30–120  $\mu$ M) was combined with teicoplanin (MBC in monotherapy 4–32 mg/L) against *S. aureus*. LL-37 synergy was observed in combination with teicoplanin against *S. aureus*, but not against *Staphylococcus epidermidis* (Koppen et al. 2019). Increased activity against *P. aeruginosa* and *S. aureus* strains with different resistance profiles was obtained after the use of colistin or imipenem with a small amount of LL-37 (Geitani et al. 2019). Combination therapy with ciprofloxacin and LL-37 against multi-drug strains of *P. aeruginosa* isolated from wounds brought satisfactory results in the form of a lower therapeutic dose compared with monotherapy (Al-Sabagh and Ghaima 2022). The combination of polymyxin B with human cathelicidin used against *E. coli* and *P. aeruginosa* strains resulted in a lower dose of the compound needed for effective antibacterial activity (Ridyard et al. 2023). The bactericidal effect of antibiotics may exacerbate inflammation because it increases the release of endotoxin due to damage to the bacterial cell (Lepper et al. 2002). LL-37 suppresses pro-inflammatory responses by binding and neutralizing LPS (Cirioni et al. 2008), allowing for the removal of harmful effects caused by an impaired inflammatory response (Koziel et al. 2014). Stimulated dendritic cells: LPS, LTA, and flagellin in the presence of LL-37 peptide released lower amounts of IL-6, IL-12, and TNF- $\alpha$  as well as the production of NO in blood vessels was reduced (Bucki et al. 2010). The beneficial effects of LL-37 were also presented in a unique study by Li et al. (2020) in which human umbilical cord mesenchymal stem cells (hUC-MSC) were modified to express fusion peptide BPI21/LL-37 and then transplanted into CLP-induced septic mice to preserve the organ function. It was demonstrated that BPI21/LL-37 modification did not only affect the immunoregulatory capabilities of hUC-MSC, but improved their LPS-neutralizing capabilities and antimicrobial effect (Li et al. 2020).

These reports demonstrate that despite the limited antimicrobial effect in the presence of blood components, the LL-37 peptide has other functions that make it an attractive candidate in the fight against sepsis. Analogs synthesized based on the LL-37 peptide may be a promising alternative to

conventional antibiotic therapy, which is not effective against multidrug-resistant strains.

## 7.2. Sepsis of fungal etiology

Fungal sepsis carries a higher risk of mortality than bacterial sepsis. The most common fungal pathogen isolated from blood: *Candida albicans* and other fungi of the *Candida* spp. (Duggan et al. 2015) are responsible for over 20% of all bloodstream infections (Holt and Nett 2024). In the United States, *Candida* is responsible for the mortality of over 40% of patients with nosocomial bloodstream infections (Memariani and Memariani 2023). Reduced immunity, presence of intravenous catheters, mucosal barrier disorders, diabetes, neutropenia, dialysis, chemotherapy, and long-term antibiotic therapy (Rapala-Kozik et al. 2015), all predispose to infections of fungal etiology. The developing resistance to available therapeutic agents and the formation of biofilms constitute a significant clinical problem in the elimination of these opportunistic pathogens that are part of the microbiota of the gastrointestinal tract, oral cavity, and vagina (Durnaś et al. 2016). Growth inhibition and killing of various strains of *C. albicans* from the American Type Culture Collection and clinical strains incubated in various experimental conditions (temperature, incubation time, medium used) were observed after the use of LL-37 at the concentration range of 0.8–64  $\mu$ M (Memariani and Memariani 2023). MIC values for 10 clinical strains of *Candida auris* incubated at 37°C for 24 h were in the range of 25–100  $\mu$ g/mL, and the minimum fungicidal concentration was 50–200  $\mu$ g/mL. Additionally, the combination of amphotericin B or caspofungin with LL-37 showed a synergistic effect against all tested strains, and the combination of LL-37 with fluconazole had synergistic effect against eight tested strains (Rather et al. 2022). Scarsini et al. (2015) tested the LL-37 peptide against *Candida* strains isolated from patients with vaginitis. The determination was performed in Sabouraud Dextrose Broth, and incubation lasted for 48 h at 30°C. The MIC for 3 strains of *Pichia kudriavzevii* (formerly *Candida krusei*), 1 strain of *Candida norvegensis* and 1 strain of *Candida parapsilosis* was in the range 4–64  $\mu$ M and for 7 strains of *Candida glabrata* and 11 strains of *C. albicans* the MIC was >64  $\mu$ M (Scarsini et al. 2015). The antifungal activity of LL-37 is pH and salt dependent and may vary in body fluids such as saliva, urine, pus, and plasma (all these fluids reduce the effect of the peptide). This observation forces the design of new synthetic peptide-based compounds to retain appropriate bactericidal activity under conditions equivalent to the human physiological environment. In such aspect, RK-31 and KS-30 peptides, being shortened forms of LL-37, were demonstrated as displaying potent antifungal activities under experimental conditions that mimic sweat. It was also demonstrated that upon membrane disruption of yeast

exposed to the peptide cell adhesion to biotic and abiotic surfaces is reduced, preventing biofilm formation (Memariani and Memariani 2023).

## 8. Conclusions

Sepsis in hospitalized patients is often caused by a selected population of multidrug-resistant microorganisms present in the hospital environment, undoubtedly contributing to high mortality. Growing antibiotic resistance and complex disturbances of physiological processes occurring in the body during sepsis require a new approach to the treatment of sepsis, which must focus not only on eliminating the etiological factor of sepsis, but also on restoring the immunological balance. LL-37 peptide exhibits antimicrobial activity against a wide range of microorganisms, and combining it with antibiotics results in a lower therapeutic dose while reducing cytotoxic effects. The peptide's ability to neutralize the effects of endotoxins, to inhibit the activation of inflammatory processes, regulating endothelial function by stiffening and reducing permeability, and acting as a hemostatic agent increases its potential for use during sepsis treatment. Because blood LL-37 concentrations change during the progression of sepsis, monitoring blood LL-37 levels may be useful for early identification of patient subgroups to establish a clinical diagnosis. Antibiofilm activity makes it possible to combat chronic infections that are resistant to many available antibiotics. The pleiotropic functions of LL-37 peptide make it an attractive candidate, but the efficacy of this peptide and its *in vivo* safety should be thoroughly investigated in the future. Due to species differences, special caution should be exercised when interpreting the results of preclinical studies on the efficacy of the LL-37 peptide obtained using animal models. The limitations of the LL-37 peptide also include high synthesis cost, cytotoxicity to host cells, and lower activity in purulent body fluids. The development of synthetic analogs with optimized biological activity based on this peptide sequence may be a promising alternative.

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## Author Contributions

All authors have read and agreed to submit the manuscript for publication.

## Conflicts of Interest

The authors declare no conflicts of interest.

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