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Review Article

Effector function and neutrophil cell death in the severity of sepsis with diabetes mellitus

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Abstract

Sepsis, a life-threatening condition resulting from immune dysregulation, is typically triggered by bacterial infections and commonly coexists with diabetes mellitus. Neutrophils are the first responders to infection and require regulated activation to control pathogen and damage-associated molecular patterns. Dysregulation of neutrophil activation leads to uncontrolled inflammatory responses, often observed in both sepsis and diabetes patients. Neutrophil dysregulation, characterized by effector dysfunction and inadequate cell death processes, can serve as a biomarker for assessing sepsis severity, particularly in diabetic patients. This review provides information on the relationship between effector function, neutrophil cell death, and the severity of sepsis in individuals with diabetes mellitus, aiming to shed light on the mechanisms underlying sepsis progression. Topics covered in the review include an overview of effector function of neutrophil cells, mechanisms of neutrophil cell death, and dysregulation of effectors and neutrophil cell death processes in sepsis severity with diabetes mellitus.

Keywords: Sepsis, diabetes, neutrophil, effector function, cell death

Introduction

Sepsis is defined as a serious, life-threatening condition in which there is deviation and dysregulation of the body's immune response to stress (trauma, burns, infection, inflammation) accompanied by organ dysfunction [1]. The most common cause of sepsis is bacterial infection [2]. Approximately 17% of sepsis cases are accompanied by comorbid diabetes [3]. Diabetes affects the entire body's metabolism, thereby increasing the severity of sepsis [4]. Sepsis has a high incidence, mortality and medical costs, making it a global health problem [5]. Severity of sepsis is grouped according to the presence or absence of tissue perfusion disorders or shock [6].

In diabetes, cells experience stress and damage because they are unable to regulate glucose transport in conditions of hyperglycemia [7]. Intracellular hyperglycemia causes cell damage caused by repeated acute abnormal changes in cell metabolism that are still reversible or persistent cumulative accumulation of macromolecules [8]. This situation causes irreversible damage even though the extracellular state is euglycemic. Cell damage in diabetes is also influenced by insulin resistance [9]. The pathogenesis of cell damage in diabetes is referred to as memory hyperglycemia where not only cell organelle damage occurs but DNA damage also occurs which causes cell properties to change permanently [10].

Neutrophils are one of the natural immune cells that work quickly and are the first to respond when an infection occurs in the body [11]. Neutrophils have an effector role as professional phagocyte cells in eliminating pathogens that enter the body [12]. Neutrophil cells also have granules that can be released to eliminate the extracellular pathogens [13]. All these

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abilities are very useful for fighting incoming pathogens. Normally and in a regulated manner, neutrophils will undergo the normal death process of apoptosis or autophagy after the pathogen can be controlled [14]. Dysregulation of neutrophil responses results in inadequate effector responses to eliminate pathogens or increase damage to the host [15]. In addition, an excessive and ongoing neutrophil response even though the pathogen has disappeared is a trigger for systemic inflammation and tissue damage in sepsis and diabetes [16].

The large number of neutrophil cells in the bloodstream and easy sampling make neutrophils could be used as interesting biomarker for analysis in the event of sepsis with or without diabetes [17]. This review addresses several clinical problems, including sepsis, a life-threatening condition characterized by immune dysregulation and organ dysfunction, commonly triggered by bacterial infection, and exacerbated by comorbid diabetes, which intensifies cellular stress and damage due to hyperglycemia and insulin resistance. Additionally, it highlights the dysregulation of neutrophil responses, leading to inadequate pathogen elimination or increased host damage, particularly in the context of sepsis and diabetes. Furthermore, this review discusses the potential use of neutrophils as biomarkers for analyzing sepsis, especially in cases involving diabetes, indicating the need for further investigation into this area to better understand and manage sepsis severity in diabetic patients.

Effector function of neutrophils

Literature indicate that neutrophils express a wide repertoire of pattern recognition receptors (PRRs) respond dynamically to stimulation during infection and inflammation, and that neutrophil PRRs serve as key regulators of host immune responses in vivo [18]. This receptor can identify pathogen associated molecular patterns (PAMPs) such as peptidoglycan and lipopolysaccharide found in bacterial cell walls [19]. Apart from that, PRR can also recognize danger associated molecular patterns (DAMPs) such as high mobility group protein B1 (HMGb1), uric acid, ATP which is released from damaged or necrotic cells during inflammation [20]. The introduction of PAMPs and DAMPs will produce intracellular signals that are expressed through the effector function of neutrophil cells [21]. The effector function of neutrophils is divided into the function of migration from the circulation to the tissue where the pathological process takes place and the immunological function of the cell to eliminate pathogens [22].

The effector functions of neutrophil migration from the circulation to the tissue where the pathological process takes place include (1) margination or neutrophil cells attaching to capillary walls, (2) diapedesis or changes in the shape of neutrophil cells through the gaps between endothelial cells, and (3) chemotaxis or migration to the tissue source of the pathogen [23]. The immunological effector functions of neutrophil cells in eliminating pathogens include (1) phagocytosis and activation of bactericidal mechanisms, (2) degranulation or release of cytotoxic products, (3) release of pro-inflammatory chemoattractant molecules or other immune stimulants such as cytokines [24].

Neutrophils are one of the professional phagocytic cells besides monocytes, macrophages, dendritic cells and osteoclasts. Phagocytosis is a cellular process to engulf and eliminate microorganisms, foreign substances and apoptotic cells whose diameter is greater than 0.5 um [25]. Particles that are swallowed during phagocytosis enter organelles called phagosomes. These phagosomes combine with lysosomes to become phagolysosomes which can degrade ingested particles [26]. Neutrophil receptors in recognizing non-self particles are through opsonic receptors and non-opsonic receptors on the plasma cell membrane. Some non-opsonic phagocyte receptors on neutrophils are N-formyl-methionyl-leucyl-phenylalanine (f-MLP) receptors, cytokine receptors such as TNF alpha and Interleukin. Opsonic phagocyte receptors on neutrophils have a stronger affinity than non-opsonic phagocyte receptors [27].

Opsonic phagocyte receptors on neutrophils are divided into immunoglobulin receptors IgG, IgA and complement receptors [28]. CD64 neutrophils are neutrophils that express receptor 1 for the Fc region of immunoglobulin G (FcyR1). FcyR1 has a high affinity for immune complexes of IgG molecules with specific antigens. Neutrophils do not express FcyR1 in the inactive state and neutrophil CD64 increases when activated by bacterial product stimuli, cytokines and inflammatory mediators, especially in sepsis [29].

There are four groupings of granule classes based on neutrophil maturase: (1) azurophilic/primary granules appear at the myeloblast and promyelocyte stages; (2) secondary or specific granules appear at the myelocyte and metamyelocyte stages; (3) gelatinase/tertiary granules produced at the stab stage; and (4) secretory granules found in the neutrophil segment [30]. Degranulation or release of granules when neutrophils are activated follows a patterned sequence. Secretory granules containing many adhesion molecules are the first granules released during neutrophil degranulation. Furthermore, gelatinase granules and specific granules are released for cell migration and create a pro-inflammatory environment and azurophilic granules which contain many antimicrobial components are released when they are at the site of inflammation [31].

The degranulation process is grouped into endocytic (intracellular) and exocytic (extracellular) [32]. Intracellular degranulation processes are often found in the process of eliminating pathogens that have been phagocytosed and united into phagolysosomes. This intracellular degranulation process is closely related to autophagy which will be discussed in cell death and the release of extracellular vesicles (ECV) [33]. Extracellular degranulation can be encased in vesicles such as ECV or without being encased in vesicles such as neutrophil extracellular trap (NET) [34]. Apart from being a medium for delivering effector molecules resulting from the phagolysosomal autophagy process, ECV also acts as a non-inflammatory cell death process. ECV can contain various molecules such as cytosolic proteins, nucleic acids, fats, membrane-associated proteins and cell membranes. ECV neutrophils can be grouped into 2 types based on their formation mechanism, namely neutrophil derived TRAILs (NDTR) which are released by neutrophils when migrating from the bloodstream to tissue and neutrophil derived microvesicles (NDMV) which are formed when they are in the focus of inflammation [35]. NET degranulation is also grouped into the vital effector function of NETosis and the suicidal cell death process of NETosis [36].

Neutrophil cell death

Normal neutrophils have a short life span with a half-life of 18-19 hours. Neutrophil cells contain many granules, enzymes, proteins and other components that can cause inflammation if cell death occurs by lysis. Normal neutrophil death must be regulated so that not to cause inflammation and damage to surrounding tissue. The process of death of normal neutrophil cells that does not cause inflammation through the apoptosis pathway, pathogen induced cell death (PICD) or efferocytosis and autophagy. Dysregulation of neutrophil cell death causes inflammation due to cell lysis through the mechanisms of necrosis, necroptosis, pyroptosis and NET-osis [37].

Cell death in neutrophils is divided into non-inflammatory and pro-inflammatory. Non-inflammatory neutrophil cell death is programmed cell death that occurs when neutrophils undergo the aging process, when neutrophils phagocyte pathogens (pathogen induced cell death/PICD) and when neutrophils experience intracellular changes due to pathogen escape or non-functioning of the phagolysosomal mechanism. The processes that occur in programmed cell death are apoptosis and efferocytosis by macrophages. Apart from non-inflammatory cell death, there is also pro-inflammatory cell death through necrosis, necroptosis, pyroptosis and NET-osis pathways. This pro-inflammatory neutrophil cell death is characterized by cell lysis so that granules and cell contents exit extracellularly and cause inflammation, tissue and organ damage, blood clots and autoimmunity [38].

Neutrophil effector function and neutrophil cell death in diabetes mellitus

Diabetes mellitus is a chronic metabolic disease characterized by hyperglycemia with various complications such as neuropathy, nephropathy, retinopathy and an increased risk of cardiovascular disease. The condition of hyperglycemia in diabetes is associated with an increased risk of infection in patients due to abnormalities in the function of leukocytes. The patient's infection profile also influences the state of glycemic control in diabetes patients [39].

Hyperglycemia in diabetes disrupts cell metabolism repeatedly and forms persistent molecular changes in cells resulting in cell dysfunction and dysregulation (**Figure 1**). Cell metabolism in hyperglycemia through the polyol pathway, hexosamine pathway, protein kinase C (PKC) pathway and advanced glycation end products (AGE) pathway increases reactive oxygen species (ROS) in mitochondria. High levels of ROS in hyperglycemia conditions initiate DNA damage through modification of polymers of adenosine diphosphate ribose (PARP). PARP will break down NAD+ into nicotinic acid (NA) and ADP-ribose (ADPR). ADPR will accumulate to form glyseraldehyde 3 phosphate dehydrogenase (GAPDH) polymers which can enter and leave the cell nucleus and interfere with the DNA repair process [40]. Detailed schematic of neutrophil effector function and cell death in diabetes mellitus is presented in **Figure 1**.

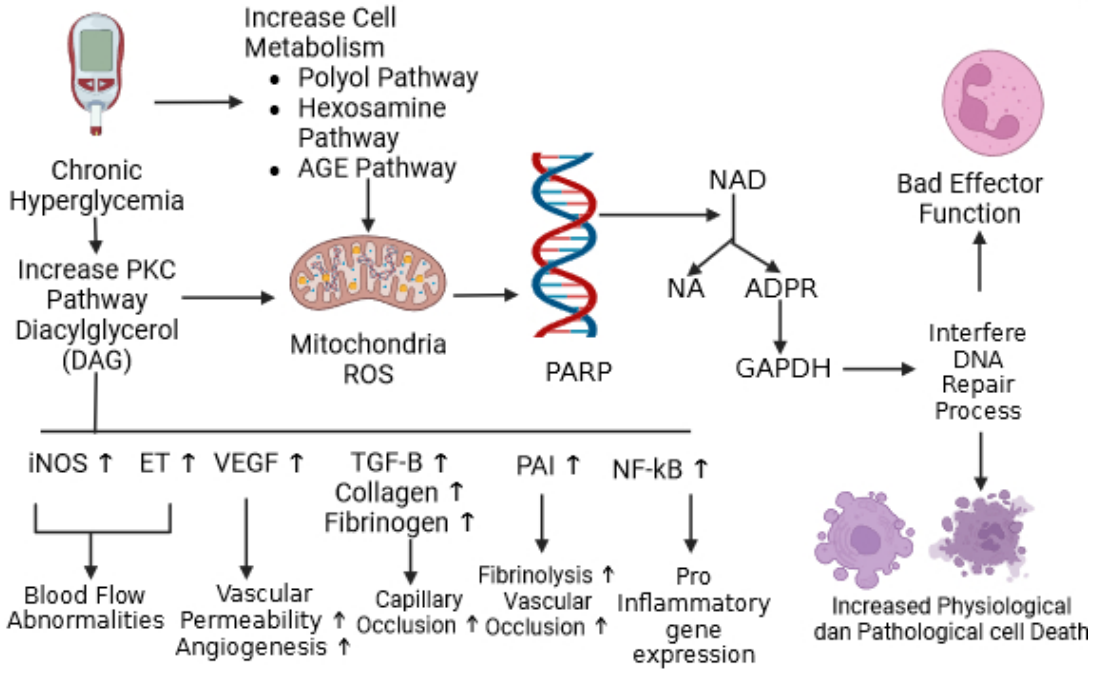

Figure 1. Neutrophil effector function and cell death in diabetes mellitus.

In the abnormal metabolism of the PKC pathway, hyperglycemia in cells has many systemic effects. The process of systemic abnormalities in the PKC pathway shows that intracellular hyperglycemia increases the synthesis of diacylglycerol (DAG) molecules which are important cofactors of PKC beta and gamma isoforms. PKC is an important component that has an effect on various gene expressions including: (1) decreasing the expression of endothelial nitric oxide syntethase (e-NOS) and increasing endothelin-1 (ET1) which causes blood flow abnormalities; (2) increasing the expression of vascular endothelial growth factor (VEGF) which causes increased vascular permeability and angiogenesis; (3) increased expression of tumor growth factor (TGF) beta, production of collagen and fibronectin which causes capillary occlusion; (4) increased expression of plasminogen activator inhibitor-1 (PAI-1) which causes a decrease in fibrinolysis and vascular occlusion; (5) increased expression of the pro-inflammatory gene nuclear factor kB (NF-κB); and (6) increased NADH oxidase which increases ROS with symptoms of multiple inflammation [40].

Neutrophil dysfunction in diabetes includes abnormalities in neutrophil migration at sites of inflammation and abnormalities in the response to elimination of pathogens. In diabetes with uncontrolled blood glucose levels, the expression of CXCL2/IL8 receptors, platelet activating factor (PAF), and fMLP which are important for migration function is disrupted [41]. The response of neutrophils to chemoattractants is reduced so that neutrophils tend to collect (swarming) on the endothelial walls of blood vessels [42]. In hyperglycemia, the phagocytic function of neutrophils decreases with high calcium concentrations and low intracellular ATP levels. The neutrophil degranulation response in the presence of bacterial endotoxin in hyperglycemia is lower than in normal blood glucose levels. Neutrophils in diabetes patients also

produce more proinflammatory cytokines and ROS even in the absence of external stimulation [43]. Cell death increases with hyperglycemia. Increased ROS, methylglyoxal (MGO) and AGEs increase signal transduction of cell death. Cell death pathways can be through death without inflammation (apoptosis, autophagy) and death with inflammation (necrosis, necroptosis, pyroptosis and suicide NET-osis) [44].

Neutrophil effector function and the process of neutrophil cell death in sepsis

Neutrophils are produced in bone marrow via granulopoiesis. In sepsis, there is an increase in the number of circulation neutrophils due to hypermigration of neutrophils from bone marrow to the circulation (**Figure 2**). At the beginning of sepsis, the neutrophils in the circulation are competent neutrophils equipped with surface receptors, granules and high maturity. These early neutrophils are called normal density neutrophils. Normal density neutrophils function normally and are able to respond well according to the conditions of the microenvironment. Normal density neutrophils have good regulation of migration effector functions, pathogen elimination effector functions and cell death processes [45].

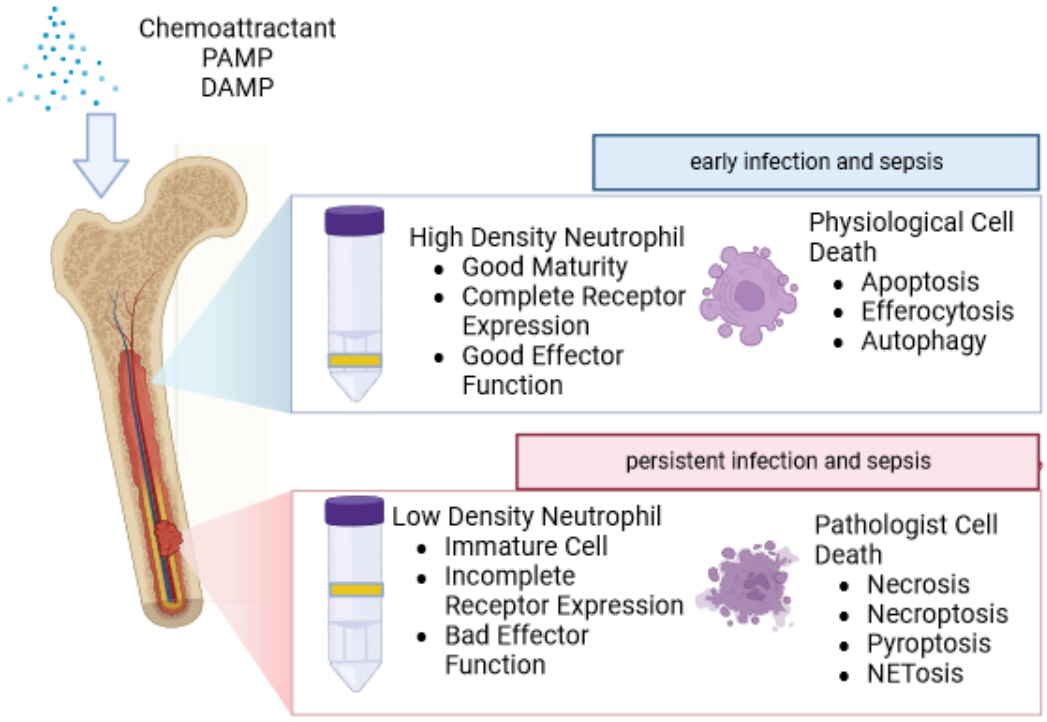

Figure 2. Neutrophil effector function and cell death in sepsis.

A continuous and uncontrolled sepsis process will produce low density neutrophils that are not yet fully mature (**Figure 2**). These low-density neutrophils are pro-inflammatory neutrophils which are characterized by increased expression of IFN-γ and TNF-α, increased endothelial cytotoxicity and reduced phagocytic capacity. Low density neutrophils consist of cells with characteristics such as myelocytes or stab formations. The nuclei of low density neutrophil cells are different from the nuclei of normal neutrophil cells which are multilobulated. The migratory ability of low density neutrophils is greatly reduced due to low CXCR2 expression resulting in the accumulation of swarming neutrophils on the blood vessel endothelium. The pathogen elimination function decreases at low neutrophil density with reduced degranulation and phagocytic function. The process of low density neutrophil cell death is prolonged and has an inflammatory pattern with necrosis, necroptosis, pyroptosis and NET-osis processes [46].

Dysregulation of effectors and neutrophil cell death processes in sepsis severity with diabetes mellitus

According to the 2016 Sepsis 3 Classification, the severity of sepsis is divided into sepsis and sepsis shock [1]. The criteria for severe sepsis are no longer used. Sepsis shock has different characteristics from hypovolemic shock, cardiogenic shock or obstructive shock. The difference between septic shock and other shocks is the occurrence of pathological vasodilation which is seen in the warmth of the extremities. Another type of shock has vasoconstriction characteristics, causing coldness in the extremities. Pathological vasodilation in septic shock is induced by nitric oxide (NO) as the main mediator that causes relaxation of vascular smooth muscle cells. NO is produced by nitric oxide synthetase (NOS) which is abundant in blood vessel endothelium. NO production in vascular endothelium is induced by cytokines or products of infectious pathogens [47]. Neutrophils, as the body's foremost and most abundant defense, can also produce NO in conditions of sepsis and diabetes [48].

Neutrophil dysregulation indicates an imbalance between stimulus and response in sepsis and diabetes. The threat of PAMP and DAMP in the patient's body is a stimulus for the neutrophil response. In a state of activated and regulated neutrophils, the neutrophil response will follow the amount of PAMP and DAMP stimulus. Dysregulation of neutrophil activation occurs if the PAMP and DAMP stimulus is high, but the neutrophil response is low as presented in **Figure 3**. Another model of neutrophil dysregulation is the neutrophil response which remains high even though the PAMP and DAMP stimulus is low [49].

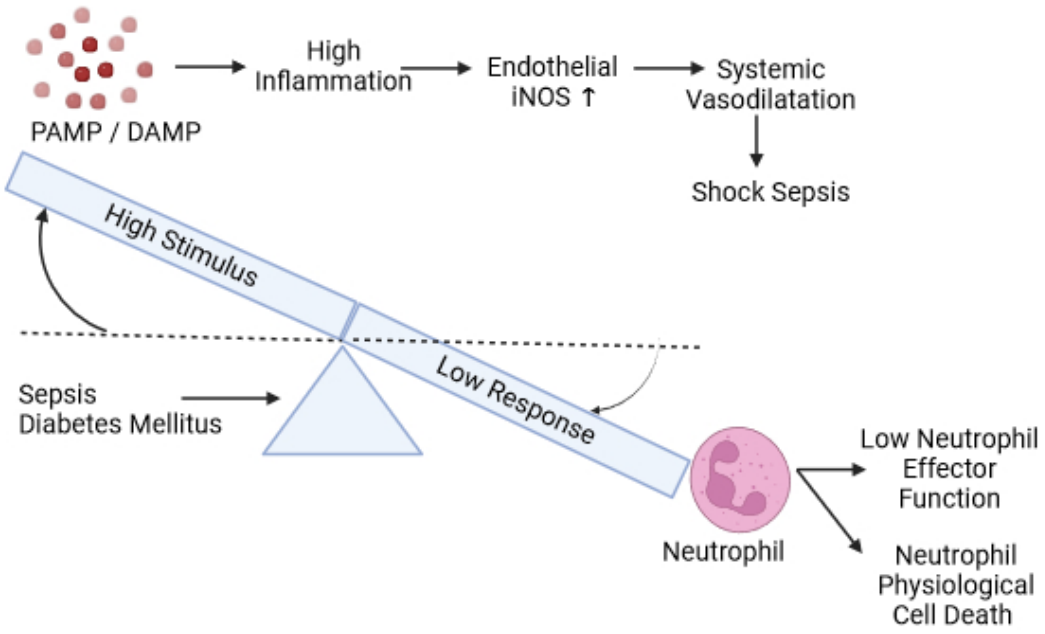

Figure 3. Dysregulation neutrophil response with high pathogen associated molecular patterns (PAMP) and danger associated molecular pattern (DAMP) stimuli.

Dysregulation of neutrophils that recognize PAMP and DAMP stimuli through PRRs does not respond appropriately can be seen in **Figure 3**. High PAMP and DAMP stimuli produce low neutrophil effector function responses, including phagocytosis, degranulation and oxidative burst, so that PAMP and DAMP are uncontrolled and stimulate endothelial iNOS expression which causes endothelial smooth muscle vasodilation and septic shock. The cell death response occurs more physiologically through the pattern of apoptosis and autophagy compared to the pathological cell death response through the pattern of necrosis, necroptosis, pyroptosis and NET-osis [50].

Dysregulation of neutrophils that overreact even though PAMP and DAMP stimuli are no longer recognized by PRR can be seen in **Figure 4**. The high activity of phagocytosis, degranulation and oxidative burst effectors in neutrophils as well as pathological cell death responses such as necrosis, necroptosis, pyroptosis and NET-osis are more dominant than

physiological cell death responses through apoptosis and autophagy, resulting in excessive inflammatory responses and tissue damage. In this condition of excessive inflammation, neutrophils will produce iNOS massively and septic shock occurs [51].

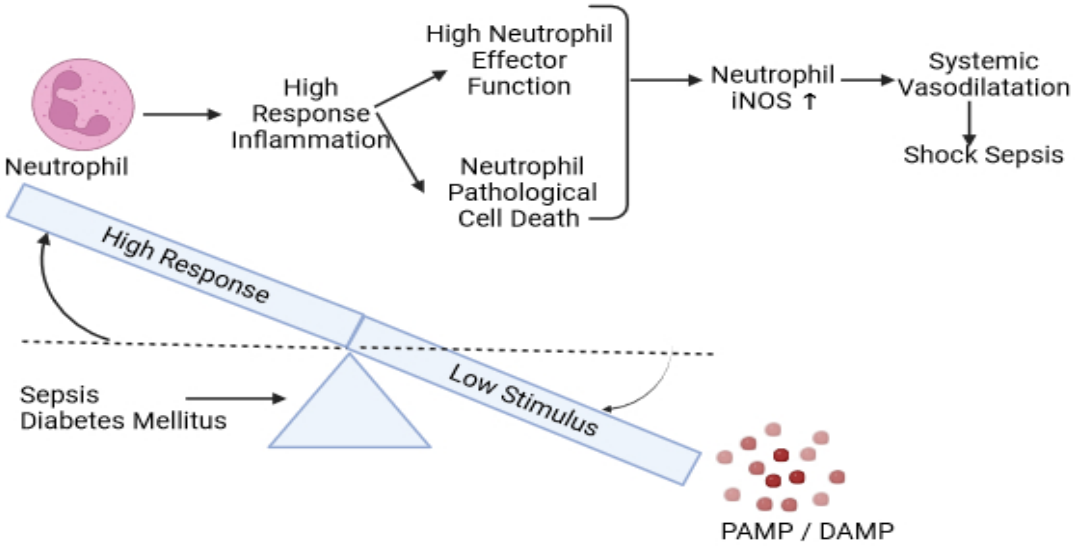

Figure 4. Dysregulation neutrophil response with low pathogen associated molecular patterns (PAMP) and danger associated molecular pattern (DAMP) stimuli.

Conclusion

Neutrophils are key players in the immune response, detecting and combating pathogens through various mechanisms including migration, phagocytosis, and release of inflammatory molecules. However, dysregulated cell metabolism in hyperglycemia leads to persistent molecular changes, impairing leukocyte function and increasing susceptibility to infections. Neutrophil dysfunction in diabetes exacerbates inflammation and tissue damage, with impaired migration, reduced pathogen response, and altered cell death pathways. In sepsis, dysregulation of neutrophil activation further complicates the immune response, leading to excessive inflammation and tissue damage, ultimately culminating in septic shock. Understanding these dysregulated processes is crucial for managing sepsis severity, especially in patients with diabetes mellitus. Future research should focus on unraveling the intricacies of neutrophil dysfunction in diabetes and sepsis, emphasizing the dysregulation of neutrophil activation and effector functions. Exploring specific pathways involved in neutrophil migration, phagocytosis, and cell death within the context of hyperglycemia and sepsis could offer valuable insights into potential therapeutic targets.

Ethics approval

Not required.

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Competing interests

All the authors declare that there are no conflicts of interest.

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Underlying data

All data are available as part of the article.

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