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Perspective

Optimal strategy for treatment of sepsis based on the host inflammatory reaction and immune response

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Introduction

Sepsis is a common complication following infections, burns, trauma, and major operations and has become the main cause of death in non-coronary intensive care units. It was the most common reason for hospitalization (except in live born infants) in 2017, with 48.9 million cases reported worldwide.[1] A study found that sepsis led to 11.0 million deaths (which represented 19.7% of all global deaths) and has required the expenditure of 38.2 billion dollars for medical care.[1]

The definition of sepsis had emerged from the consensus meeting held by the American College of Chest Physicians and Society of Critical Care Medicine in 1992. It was first defined as a systemic inflammatory response syndrome (SIRS) caused by infection (sepsis-1). The standard definition facilitates patient selection for clinical therapeutic trials and allows effective communication among investigators. Although the criteria for SIRS demonstrate adequate sensitivity, they have shown poor specificity over the past few decades. In addition, clinical trials targeting inflammatory cytokines have failed to demonstrate benefit in these cases; this has led to uncertainties regarding the nature of sepsis. In 2016, sepsis was redefined as life-threatening organ dysfunction caused by infection-induced dysregulation of the host response (sepsis-3).[2] The new definition has shifted the focus from an inflammatory reaction to severe organ dysfunction and emphasizes the underlying mechanisms with regard to abnormal host regulation. In this context, various systems are involved in the host response that maintains homeostasis; this includes the immune network, coagulation system, and neuroendocrine axis. Notably, the evolution of the definition of sepsis indicates a profound understanding of its pathophysiological process. Infection-induced inflammation plays an important role in the development of sepsis, and its regulation is largely dependent on the immune response.

Inflammatory Reaction in Sepsis

Inflammation is a defensive host reaction that is triggered by noxious stimuli. The concept of inflammation is defined based on pathological characteristics including redness, swelling, heat, pain, and subsequent loss of organ function. These pathological disorders are caused by increased permeability of blood vessels, migration of fluid and proteins, and recruitment of leukocytes to the site of infection or damaged tissue.

Inflammation aims to localize and eliminate pathogens and injurious agents and to remove damaged tissue components to promote wound healing. However, excessive inflammation may occur in many diseases and is typically seen in sepsis. In cases of infection or tissue injury in the host, the common component of invading microorganisms (known as pathogen-associated molecule patterns [PAMPs]) is recognized by pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs), nucleotide-binding oligomerization domain-like receptors (NLRs), C-type lectin receptors, and absent in melanoma 2-like receptors.[3]

Although PRRs are distributed widely, they are mainly found on innate immune cells including macrophages, neutrophils, dendritic cells (DCs), eosinophils, basophils, and mast cells and are able to rapidly respond to pathogen invasion. Cell death-mediated formation of intracellular molecules (known as damage-associated molecular patterns [DAMPs]) can also be recognized by PRRs; these include high mobility group box-1 (HMGB1) protein, mitochondrial deoxyribonucleic acid,

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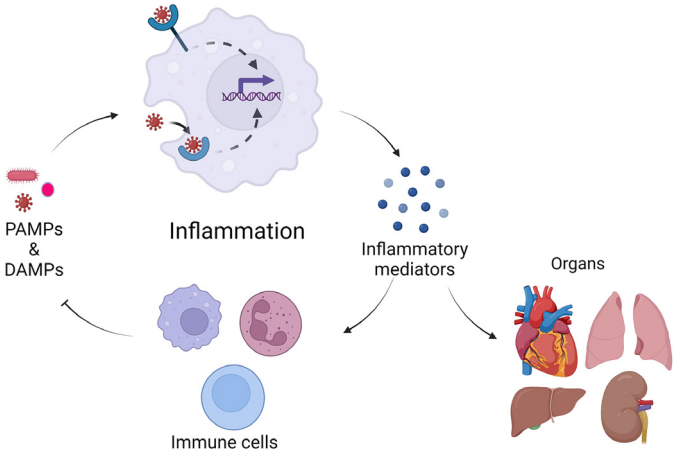

Figure 1. Inflammatory reaction in sepsis. During the course of an infection, PAMPs and DAMPs are recognized by widely distributed PRR-expressing cells including immune, endothelial, and other types of cells. PRR activation triggers the induction of various inflammatory mediators, including chemokines, cytokines, vasoactive amines and peptides, complements, lipid mediators, and proteolytic enzymes, among others. These mediators transduce inflammatory signals to immune cells and promote their activation to eliminate pathogens. Inflammatory mediators can also affect the function of multiple organs. Although the organs do not directly participate in the defense against pathogens, they attempt to maintain homeostasis in the host by adapting to the inflammatory response.
DAMPs: Damage-associated molecular patterns; PAMPs: Pathogen-associated molecule pattern; PRR: Pattern recognition receptor.

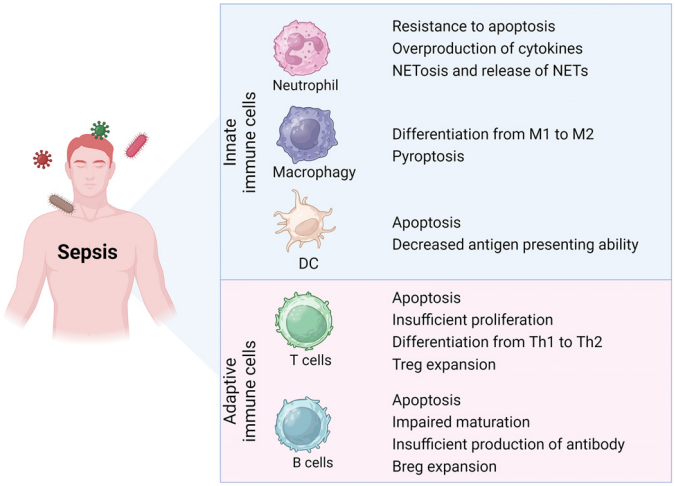

Figure 2. Effect of sepsis on immune cells. In the setting of sepsis, both innate and adaptive immune cells exhibit alternations in phenotype and function. The details are described in the main text.
DC: Dendritic cell; NETs: Neutrophil extracellular traps.

and heat shock proteins, among others. Upon specific recognition of PAMPs/DAMPs, the relevant PRRs activate intracellular signals to regulate the production of mediators including cytokines, chemokines, vasoactive amines and peptides, complements, lipid mediators, and proteolytic enzymes.[4] These mediators subsequently transmit inflammatory signals to their targets, which involve various host tissues and organs. In this context, non-effective targets are affected by inflammatory signals, but they do not directly participate in the elimination of pathogens. These tissues and organs attempt to maintain homeostasis in the host by adapting to inflammation via various mechanisms, which include an increase in cardiac output, vasodilatation, and acceleration of metabolism, among others. Conversely, immune cells serve as an effective target of inflammatory signals, which provide negative feedback to eliminate pathogens[5] (Figure 1).

Lipopolysaccharide (LPS) represents a major component of the cell wall in Gram-negative bacteria and is a common PAMP that triggers sepsis. Extracellular LPS conjugates with CD14 and is transferred to the myeloid differentiation protein 2-TLR4 complex. Dimerized TLR4 transduces intracellular signals to stimulate the production of pro-inflammatory cytokines via myeloid differentiation protein 88-dependent or Toll or interleukin (IL)-1 receptor domain-containing adapter-inducing interferon (IFN)-β-dependent pathways. The former activates nuclear factor-κB, subsequently inducing IL-1β, tumor necrosis factor (TNF)-α, and IL-6; the latter is triggered by internalization of the TLR4/Toll or IL-1 receptor domain-containing adapter-inducing IFN-β complex into endosomes. This further promotes the transcription of type I IFN and the activation of mixed lineage kinase domain-like pseudokinase-dependent necroptosis. In addition to transcriptional regulation of pro-inflammatory cytokines, TLR4 activation and its associated intracellular signaling pathways appear to be essential for inflammasome protein transcription; it is also involved in coupling during inflammasome assembly, which results in cell pyroptosis. Excessive TLR4 activation is believed to be responsible for the cytokine storm and consequent occurrence of sepsis. However, outer membrane vesicles extruded by bacteria enable cytosolic localization of LPS, which is TLR4-independent.[6] Intracellular LPS directly activates caspase-11 in mice or caspase-4/5 in humans; this cleaves gasdermin-D to form membrane pores and leads to pyroptosis. The production and secretion of pro-inflammatory cytokines may be augmented by either TLR4-dependent or -independent pathways to initiate an inflammatory reaction. In addition to LPS, the widely distributed PRRs recognize other PAMPs (including flagellin protein, peptidoglycan, lipoteichoic acid, and nucleic acids of both bacteria and viruses) and trigger intracellular signaling.

Among the DAMPs involved in the pathogenesis of sepsis, HMGB1 (a chromatin-binding protein) is the most studied. Following extrusion from the cell, it is recognized by receptors for advanced glycation end products as well as TLR4. Extracellular HMGB1 also binds to LPS and facilitates its transport into the cytoplasm via receptors for advanced glycation end products. It additionally binds to other molecules and cytokines such as deoxyribonucleic acid, ribonucleic acid, histones, nucleosomes, IL-1α, and IL-1β to form heterocomplexes that are recognized by PRRs. HMGB1 may therefore act as a carrier for other DAMPs in addition to being a pro-inflammatory mediator.[7]

Immune Response in Sepsis

Immune cells are the major source of inflammatory mediators and function as effective targets of inflammatory signals. The immune response is therefore closely associated with the course of the inflammatory reaction. Sepsis induces widespread alterations in the population and function of both innate and adaptive immune cells (Figure 2).

Innate immune response

Neutrophils are essential for early control of invading pathogens; they concentrate at the site of infection along chemokine gradients within hours. Activated neutrophils secrete cytokines that transduce inflammatory signals. In particular, activated neutrophils undergo NETosis, whereby they capture and destroy invading microorganisms by setting neutrophil extracellular traps (NETs) and ingesting them. In the absence of activation, mature neutrophils progress to apoptosis within 7–12 h; however, PAMP-activated neutrophils are resistant to apoptosis in cases of infection. The accumulation of neutrophils leads to an abundance of pro-inflammatory cytokines, reactive oxygen species, and proteases, which participate in the cytokine storm and tissue injury. Accumulated neutrophils also trigger extensive microthrombus formation at the base of the NETs; these are known as immunothrombi. NETs formation in the alveoli impairs ventilation and directly induces acute respiratory distress syndrome (ARDS), which is one of the factors associated with mortality in sepsis.[8]

Resident macrophages act as the first sensor of invasive pathogens in tissues. Activated macrophages exhibit the M1 phenotype, which has the capacity to phagocytose and release pro-inflammatory cytokines and chemokines. However, macrophages gradually differentiate to the M2 phenotype during the course of sepsis; this phenotype typically secretes anti-inflammatory cytokines including IL-10, transforming growth factor (TGF)-β, and the IL-1 receptor antagonist (IL-1ra). Monocytes from patients with sepsis are likely to exhibit LPS tolerance, which is characterized by an impaired ability to induce pro-inflammatory cytokines (such as TNF-α, IL-1, IL-6, and IL-12) even in the presence of LPS for further stimuli *in vitro*. Conversely, the release of anti-inflammatory mediators, such as IL-1ra and IL-10, is neither impaired nor enhanced. The underlying mechanism is complicated and not fully understood; however, it may involve alterations in extracellular conditions and reprogramming of intracellular signaling. In recent years, pyroptosis of monocytes and macrophages has been found to be predominantly responsible for the overwhelming inflammatory response. Pyroptosis was originally considered to be a protective process that aimed to eliminate intracellular pathogens in the early stages of infection. However, sepsis induces excessive pyroptosis in monocytes and macrophages; this leads to the secretion of large quantities of IL-1β and the formation of other intracellular components that contribute to uncontrolled inflammation and even tissue injury or organ dysfunction.[9] In this context, studies on animal models of sepsis suggest that inhibition or genetic deficiencies of the pyogenic pathway (including caspase-1, caspase-11, and gasdermin-D) significantly improve survival.[10,11]

DCs are the most potent antigen-presenting cells; they present specific antigens to adaptive immune cells and induce their effective proliferation. They are therefore indispensable to the link between innate and adaptive immune cells. Notably, DCs are extremely sensitive to sepsis-induced apoptosis. In a postmortem study, patients with sepsis and trauma demonstrated a considerable reduction in the number of splenic DCs.[12] Those with severe sepsis also demonstrate a similar reduction in circulating DCs.[13] In a study where mice were subjected to a lethal endotoxin challenge, selective overexpression of the anti-apoptotic factor, B-cell lymphoma 2, in DCs improved the survival rate.[14] In addition to apoptosis, surviving DCs show a reduction in the expression of human leukocyte antigen (HLA)-DR, CD80, and CD86 and an increase in the levels of IL-10; this prevents the induction of effective T-cell proliferation and promotes T-cell anergy or regulatory T-cell (Treg) expansion instead. The loss of both numbers and function of DCs is considered to be a major cause of immunosuppression in sepsis.

Adaptive immune response

Adaptive immunity plays an immunoregulatory role that aims to maintain homeostasis in the host response and limit damage after infection. Advances in the management of sepsis support the concept that most patients survive the early hyperinflammatory phase but progressively or concomitantly enter into the immunosuppressive state, which leads to secondary infection and death.[15] Impairment of T cells that are involved in adaptive immunity is a major factor responsible for immunosuppression and even death in patients with sepsis.

In this context, T (especially $CD4^+$) cells are susceptible to sepsis-induced apoptosis. Reports suggest that absolute counts of $CD4^+$ T cells are obviously reduced in sepsis; non-recovery of sufficient counts lead to poor outcomes.[16] Surviving $CD4^+$ T cells subsequently exhibit exhaustion or anergy. Studies have indicated a reduction in T-cell proliferation and a shift in polarization to the Th2 phenotype. This is evident from decreased production of IL-2 and IFN-γ (from Th1 cells) and increased formation of IL-4, IL-10, and TGF-β (from Th2 populations) in patients with sepsis and murine models.[17,18] Additionally, the expression of inhibitory receptors (including programmed death-1 [PD-1]) on $CD4^+$ T cells is upregulated in patients with septic shock. This is associated with an increased risk of secondary nosocomial infections and mortality and may be attributed to the potent inhibition of cell proliferation.[19]

Treg cells exert potential immunosuppressive effects by further reducing the proliferation of effector T cells and dampening their function. Unlike effector T cells, Treg cells are resistant to sepsis-mediated apoptosis and even undergo expanded differentiation owing to increased Foxp3 expression. Clinical studies have consistently reported significantly higher Treg populations in patients with sepsis; persistently high proportions of Treg cells are associated with mortality.[20] In this context, sepsis may potentiate the immunosuppressive effects of Treg cells. In a study, splenic Treg cells from septic mice produced more IL-10 and TGF-β than naturally differentiated Treg cells from healthy controls; this further inhibited the proliferation of effector $CD4^+$ T cells.[21]

B cells have the ability to produce antibodies, which mediate humoral immunity. Recent studies have revealed additional functions of B cells, including antigen presentation and cytokine production. Similar to T cells, B cells demonstrate a decline in the number among patients with sepsis. The maturation and function of B cells are also impaired after sepsis, and their ability to secrete antibodies is reduced due to insufficient synthesis of immunoglobulins. Several studies have suggested that regulatory B-cell numbers are significantly increased in patients with sepsis; this aggravates immunosuppression owing to the secretion of IL-10 and IL-35.[22]

The adaptive immune response regulates innate immunity by providing feedback, which limits excessive inflammation. Sepsis can impair adaptive immunity and lead to the production of large quantities of anti-inflammatory cytokines (including IL-10 and IL-4); this inhibits pro-inflammatory cytokine release and leads to monocyte anergy by down-regulating major histocompatibility complex expression.

Therapeutic Targets in Sepsis

Based on the first definition of sepsis, it was suggested that compensatory anti-inflammatory response syndrome (CARS) occurs subsequent to SIRS; this aims to reduce excessive inflammation and leads to immunosuppression. Initially, SIRS and CARS were considered to occur consecutively. However, increasing evidence suggests that these two key events occur concomitantly during the development of sepsis. In their study, Xiao et al.[23] analyzed the transcriptome in circulating leukocytes from patients with severe trauma and burn injuries. They found that a global induction of gene expression was involved in both pro- and anti-inflammatory responses. These findings were supported by reports that suggested pro- and anti-inflammatory cytokines to be simultaneously affected after the onset of sepsis.[24] The balance between pro- and anti-inflammatory responses appears to be important for the elimination of pathogens and the promotion of recovery. Disturbance of the balance in favor of a pro-inflammatory state leads to tissue injury and organ failure; on the contrary, an overwhelming anti-inflammatory response leads to anergy and immunosuppression. Continuous cross-talk and fine adjustments are needed to maintain this delicate balance. The underlying pathophysiological mechanisms of sepsis are therefore not simple and linear; they involve a complicated network of multiple pathways. In this context, numerous clinical trials on the treatment of sepsis have failed over the past decade owing to a lack of comprehensive understanding of the pathophysiological nature of sepsis.

Anti-inflammatory agents

TLR4 is an important target that is essential for the recognition of LPS. Numerous studies that employed anti-TLR4 agents for treating sepsis have shown a significant reduction in cytokine production, in both animal models and *in vitro* experiments.[25,26] Eritoran, a lipid A (of LPS) mimetic, competitively binds to myeloid differentiation protein 2; it is a well-known antagonist of TLR4 in clinical trials. Unfortunately, treatments with eritoran do not reduce mortality in patients with sepsis even at high doses.[27] This unexpected failure may be attributed to the involvement of other PRRs that are triggered by various PAMPs and DAMPs. Notably, although the inflammatory reaction is sensed and triggered by TLR4, the subsequent cascades amplify signals across multiple systems that are involved in cross-talk or feedback regulation. Blockade of a single target therefore does not sufficiently improve the final outcome. In this context, anti-inflammatory agents that specifically target harmful mediators have been developed. These include anti-TNF antibodies, soluble TNF receptors, IL-1ra, and anti-prostaglandin agents, among others. Although all the agents appeared to be effective in pre-clinical trials, none have significantly improved survival rates in clinical trials.[28] Notably, uncontrolled inflammation and the cytokine storm may directly contribute to organ failure; however, targeting one of the harmful mediators cannot reverse progress (owing to the involvement of a complicated network). In particular, patients who survive the initial hyper-inflammation develop a persistent inflammatory state in conjunction with immunosuppression (due to anergy and exhaustion of immune cells).

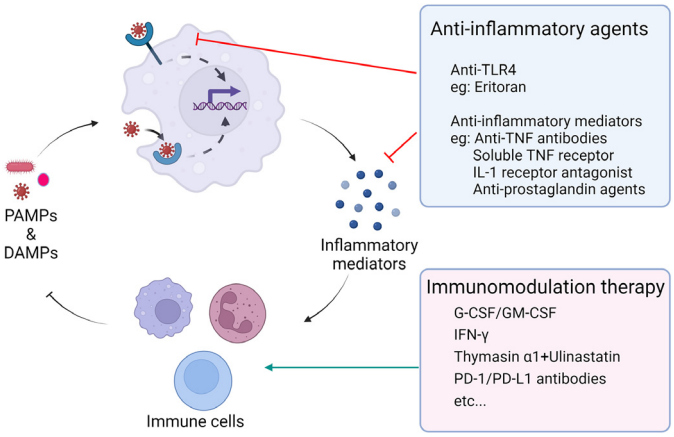

Figure 3. Therapeutic targets in sepsis. Previous intervention strategies focused on the anti-inflammatory response (such as blockade of TLR4 and anti-inflammatory mediators). In recent years, novel immunomodulation has been considered to be more effective in improving outcomes among patients with sepsis. Some immunomodulatory agents mentioned in the main text include G-CSF/GM-CSF, IFN-γ, Tα1, and antibodies to PD-1 and PD-L1.
DAMPs: Damage-associated molecular patterns; G-CSF: Granulocyte colony-stimulating factor; GM-CSF: Granulocyte-macrophage colony stimulating factor; IFN-γ: Interferon-γ; IL-1: Interleukin-1; PAMPs: Pathogen-associated molecule pattern; PD-1: Programmed death-1; PD-L1: PD-ligand 1; Tα1: Thymosin α1; TNF: Tumor necrosis factor; TLR4: Toll-like receptors 4.

Immunomodulatory therapy

Therapeutic strategies that aim to modulate the immune response have been developed in recent years (Figure 3).[29] In this context, granulocyte colony-stimulating factor (G-CSF) and granulocyte macrophage (GM)-CSF are cytokines that promote the differentiation of neutrophils, monocytes, and macrophages. Numerous clinical trials have investigated the efficiency of G-CSF and granulocyte-macrophage colony stimulating factor (GM-CSF) in patients with sepsis. However, a meta-analysis found that treatment with either G-CSF or GM-CSF did not reduce in-hospital mortality.[30] Extensive activation of neutrophils and macrophages offers no benefit in overcoming infections and may conversely induce hyper-inflammation, coagulation disorders, and tissue injury; patients with sepsis who have definite immunosuppression (especially in innate immunity) may therefore benefit from treatment with G-CSF or GM-CSF.[31]

Although IFN-γ is essential for activation of the innate immune response, it is produced in insufficient quantities during a septic episode due to T-cell anergy. In a small clinical trial, IFN-γ was administered to patients with sepsis who demonstrated low HLA-DR expression in monocytes. The findings suggested that monocyte activation was restored (as evidenced by enhanced HLA-DR expression and LPS-induced TNF-α formation). This potential efficiency of IFN-γ was supported by the

fact that eight of nine patients with sepsis had survived.[32] In a recent phase II clinical trial, critically ill patients who required mechanical ventilation were treated using IFN-γ1b. The treatment did not significantly reduce either the incidence of hospital-acquired pneumonia or the 28-day mortality rate compared to those who received placebo.[33] Owing to the heterogeneity among patients with sepsis and dynamic alterations in immune status, biomarker-guided therapy may offer more satisfactory outcomes and safety; this is especially applicable to cases where treatment aims to modulate host innate immunity.

Thymosin α1 (Tα1) is a natural thymic peptide that acts as an endogenous regulator of both the innate and adaptive immune systems. A multicenter clinical trial from China found that treatment with Tα1 improved monocyte HLA-DR expression and reduced mortality in patients with severe sepsis.[34] Combined treatment with Tα1 and the anti-inflammatory agent, ulinastatin, has been proposed to prevent the overactivation of the innate immune response. In this context, a meta-analysis that included 8 randomized controlled trials showed that combined treatment with Tα1 and ulinastatin reduced Acute Physiology and Chronic Health Evaluation II scores, the duration of mechanical ventilation, and 28-, 60-, and 90-day mortality rates in patients with sepsis.[35] The strategy of employing combination therapy has therefore proven to be reasonable; this concept may be used for the development of novel protocols.

PD-1/PD-ligand 1 (L1) has been demonstrated to have potential immunosuppressive properties as it is associated with T-cell anergy in many human diseases, including septic complications and several types of cancer. Antibodies targeting PD-1 and PD-L1 have been successfully used in the treatment of cancers. In animal experiments, blockade of PD-1/PD-1 signals by monoclonal antibodies has been found to significantly prevent sepsis-induced lymphopenia and reduce mortality.[36] Treatment of lymphocytes from septic patients with these antibodies inhibits cell apoptosis and increases the release of IL-1 and IFN-γ.[37] Hotchkiss et al.[38,39] recently evaluated the safety and tolerability of anti-PD-1 and anti-PD-L1 antibodies among septic patients in phase Ib trials. Treatment with PD-1 and PD-L1 antibodies obviously upregulated monocyte HLA-DR expression and did not increase the production of pro-inflammatory cytokines. The results demonstrated these antibodies to be safe and well tolerated in the treated septic population. Therapeutic blockade using immune checkpoint inhibitors is a more effective immunomodulatory strategy than specific reversal of T-cell anergy via down-regulation of negative signals.

Conclusions

In conclusion, the underlying pathophysiological mechanisms of sepsis appear to be complicated and involve disorders in excessive inflammation, immune dysfunction, and multiple systems. The complicated processes and various phenotypes are closely related to the heterogeneity observed in septic patients. As the inflammatory and immune responses are dynamic and change continuously in all cases, precision therapy needs to be guided by appropriate biomarkers that reflect individual immune status. Notably, modulation of single targets could lead to unexpected effects as the host response in sepsis involves a network of pathways. It is therefore evident that the optimal therapeutic strategy for septic complications needs to be considered carefully, and the approaches need to be validated in appropriately designed studies.

Author Contributions

Hui Zhang: Writing – original draft. **Ning Dong:** Writing – review & editing. **Yongming Yao:** Supervision, Funding acquisition.

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Ethical Statement

Not applicable.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

None.

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