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PERSPECTIVE

The double-edged sword of biomarkers in severe infection: Value and risks of combined detection

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1 CLINICAL CHALLENGES OF SEVERE INFECTIONS

The increasing incidence of severe infections is one of the most formidable challenges for critical care medicine, and it is increasingly common worldwide. The epidemiology shows that millions are admitted annually for sepsis and related morbidities, with age-dependent surges in the incidence of sepsis, and a higher risk among elderly patients or those with comorbid conditions [1]. Severe infections have highly variable clinical follow-up needs. Continuous development of antibiotics and intensive care units, along with early diagnosis and combined targeted treatments, still plays a key role in reducing patient mortality.

Severe infection progresses rapidly and is hard to identify, so early recognition is critical to lowering mortality. Conventional inflammatory markers are slow to respond although they are relatively sensitive to stress. Blood cultures (BC) are sensitive, and BC identification detects 50–80% of mixed infections [2, 3]. However, data from large cohorts indicate that only half of cases are accurately diagnosed, since BC often fails to identify low-virulence organisms [2]. Thus, the simultaneous detection of multi-biomarkers has attracted wide research attention to get a balance between sensitivity and specificity for optimal diagnosis and risk stratification.

This diagnostic challenge is particularly evident in vulnerable populations, such as neonates and preterm infants, in whom severe infections may progress rapidly and lead to high mortality in the neonatal intensive care unit. From a clinical perspective, early diagnosis is crucial with the requirement of simultaneous identification of platelet count and platelet indices to optimize comorbid status [4]. Combined detection of multiple biomarkers is an important means to promote early identification of infection; however, clinical application issues remain unsolved, such as lack of standardization, and the approach is risk-dependent.

2 TYPES AND FUNCTIONS OF BIOMARKERS

2.1 Classification of common biomarkers

Biomarkers of severe infection may be classified based on their origin and functions. Systemic inflammatory response is characterized by so-called pro-inflammatory cytokines, such as C-reactive protein (CRP), procalcitonin (PCT), and interleukin (IL)-6. IL-2 and IL-6 also contribute to improved diagnosis of bacterial infection in laboratory testing. Immune-related biomarkers, such as IL-10 and tumor necrosis factor-α (TNF-α), reflect host immune shifts in sepsis; elevated IL-10 indicates

immunosuppression and poor prognosis, whereas increased TNF-α suggests hyperinflammation and worse outcomes [5, 6]. Infection-related organ injury is assessed with lactate and liver/kidney function tests. All of these biomarkers can be affected by confounding factors, so the diagnostic and prognostic effects are complementary, warranting their combined use in clinical practice.

2.2 Roles of various biomarkers in diagnosis

Various infection biomarkers serve distinct diagnostic roles. Pathogen-related markers (such as PCT) identify bacterial infections and assess severity; inflammatory or immune-related markers indicate inflammatory and immune status; organ function-related markers evaluate disease progression and prognosis. Combining markers may enhance sensitivity and support early diagnosis. However, biomarker results should be interpreted alongside clinical conditions and individual characteristics to avoid overreliance on a single marker and ensure accurate diagnosis and treatment.

2.3 Research progress on emerging biomarkers

Emerging multi-omics technologies have revealed new biomarkers for severe infections, including immune checkpoint molecules, extracellular vesicle miRNAs, metabolomics, and host transcriptomics. One candidate marker is histone H3 lysine 18 lactylation, which reflects disease severity and regulates macrophage anti-inflammatory function during sepsis through arginase-1 expression and inflammatory factors [7]. For example, extracellular vesicle-derived miRNA signatures have shown high diagnostic accuracy for septic shock, with a three-miRNA model (miR-100-5p, miR-148a-3p, and miR-451a) achieving an area under the curve of 0.894 in validation cohorts [8]. These functional biomarkers reveal immune dysregulation characterized by concurrent inflammation enhancement and immune evasion, paving the way for early risk stratification, prognostic assessment, and personalized therapy. However, there is a lack of standardization, accompanied by high cost and limited accessibility.

3 ADVANTAGES OF COMBINED DETECTION

3.1 Improved sensitivity and specificity

Systemic combined detection of severe infection markers improves diagnostic sensitivity and specificity. Individually, single biomarkers fail to detect early infections due to biological variation and comorbidities. However, multi-biomarker evaluation offsets these limitations through complementary effects. For example, combined detection of IL-6 and PCT showed a sensitivity of 93.84% and a specificity of 96.72% for severe bacterial infection [9]. Thus, combined detection supports early diagnosis and individualized clinical decision-making.

3.2 Promoting the practice of individualized medicine

Multiplex detection of severe infection biomarkers reflects the inflammatory and immune status to guide individualized medicine. IL-1β, IL-6, and TNF-α are significantly elevated in sepsis-induced cardiomyopathy, and their combined detection improves diagnostic and prognostic evaluation [10]. These inflammatory factors and related parameters could be incorporated to dynamically assess infection severity and progression, thereby guiding antimicrobial therapy, identifying high-risk patients, and tailoring interventions, serving as a quantitative prognostic reference.

3.3 Improving clinical decision-making and patient prognosis

Multiplex detection improves the early diagnosis of deep infections, aiding risk stratification and therapy. Tracking IL-6, PCT, and CRP identifies high-risk patients for timely treatment and monitoring adjustments. No single biomarker is diagnostic by itself and must align with clinical data. Simultaneous detection enhances diagnostic accuracy and enables individualized management of severe infections, as shown in **Figure 1**.

4 RISKS OF OVER-RELIANCE ON COMBINED DETECTION

4.1 Potential risks of misdiagnosis and missed diagnosis

These biomarkers indicate immune dysregulation involving both inflammatory amplification and suppression, supporting early stratification, prognostic assessment, and individualized therapy. However, lack of standardization, high cost, limited availability and insufficient validation restrict clinical use. Pro- and anti-inflammatory cytokines in hypothermic sepsis are generally low, and combined detection may fail to detect these cytokines, increasing false negatives [11]. Ignoring history, signs and imaging leads to misdiagnosis and mistreatment. Thus, combination assays must be applied within the clinical context and interpreted cautiously.

4.2 Increased healthcare costs and resource waste

Multiplex biomarker detection may improve the diagnostic sensitivity for severe infections, but at a high cost. Repeated testing increases expenses, prolongs hospitalization and treatment, and consumes resources. Without specific indications, no evidence shows combined detection improves outcomes; instead, it burdens laboratories and adds cost. Hence, evaluating the economic value and clinical necessity of multiplex biomarker testing is necessary for rational and sustainable clinical management.

4.3 Risk of deviating from clinical judgment

Simultaneous evaluation of PCT, CRP, and IL-6 may improve diagnostic and prognostic assessment in severe infection and

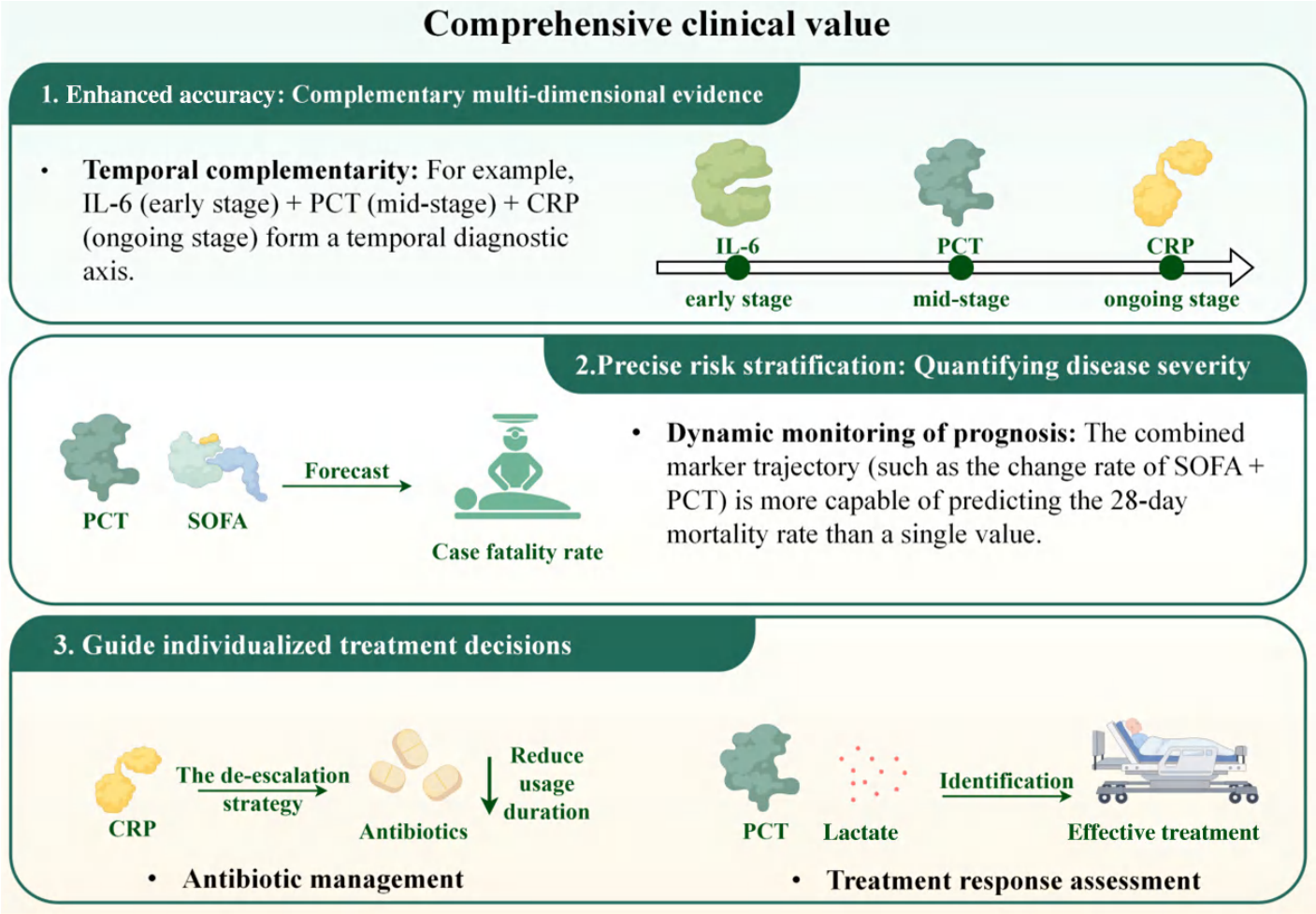

Figure 1. Schematic of the comprehensive clinical value of combined biomarker detection in diagnosis and risk stratification of severe infections. This figure visually depicts the integrated clinical value provided by the simultaneous detection of multiple biomarkers (e.g., IL-6, PCT, CRP, lactate, and the SOFA score) for the diagnosis, risk stratification, and individualized treatment guidance of patients with severe infections. The model illustrates the synergistic nature of biomarkers applied over a continuum and at specific monitoring time points for prognostic value and utility to guide antibiotic de-escalation therapy and assess its efficacy. IL-6, interleukin-6; PCT, procalcitonin; CRP, C-reactive protein; SOFA, Sequential Organ Failure Assessment.

support clinical decision-making before pathogen identification [12]. As shown in **Figure 2**, IL-6 increases earlier than PCT, a bacterial infection marker. PCT and lactate require serial monitoring with the Sequential Organ Failure Assessment score to predict shock risk. However, biomarker levels vary with age and comorbidities, and overreliance may obscure accurate assessment and cause overtreatment. Thus, biomarker detection should be integrated with symptoms, signs, and imaging to guide individualized management.

5 FUTURE DIRECTIONS AND PRACTICAL RECOMMENDATIONS

Advances in molecular diagnostics and high-throughput technologies enable precise multidimensional detection of severe infection biomarkers. Liquid biopsy, single-cell analysis, and multiplex immunoassays jointly assess multiple biomarkers, improving sensitivity and specificity. Artificial intelligence and machine learning (e.g., Light Gradient Boosting Machine, Extreme Gradient Boosting Machine, Random Forest) show strong predictive ability in sepsis-associated liver injury. Stacked ensembles enhance prediction robustness for early intervention and personalized therapy [13]. These research findings indicate that the future application of biomarkers should not be limited to the interpretation of a single biomarker, but rather should combine molecular indicators with clinical variables. Nonetheless, these emerging techniques are in need of multi-center validation and guideline development for clinical application in order to tackle issues concerning standardization, cost, and practicability.

Clinically, it is often difficult to distinguish infection-related complications solely based on traditional biomarkers. Therefore, comprehensive analysis is particularly important. Pneumonia,

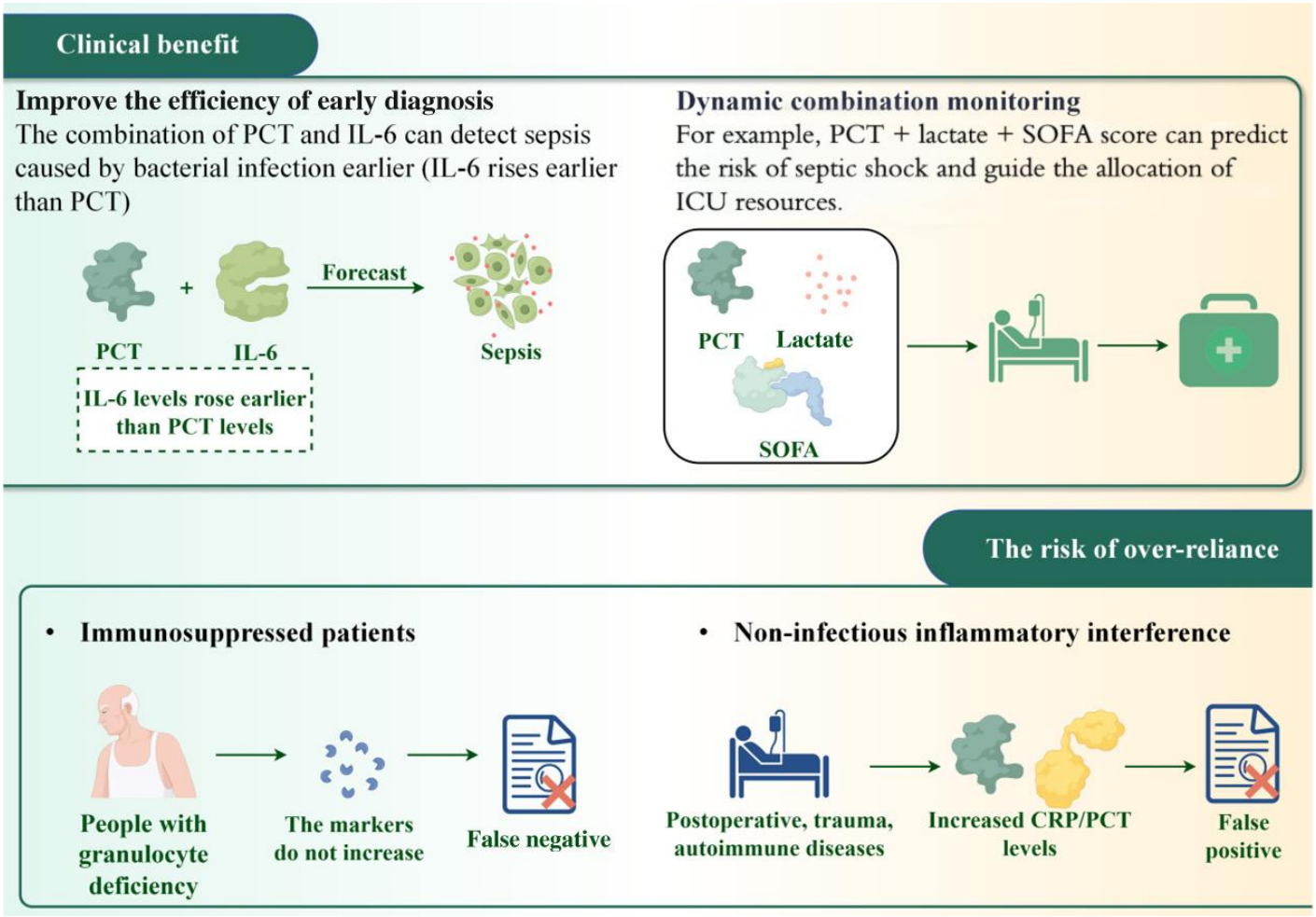

Figure 2. Clinical value and potential risks of combined biomarker detection in severe infections. This figure illustrates the clinical significance of multi-biomarker multi-signals (PCT, IL-6, lactate, SOFA) detection-based prediction in severe infections such as improving early diagnosis and dynamic monitoring and evaluating prognosis. It also highlights the potential for false negative or false positive test results in particular patient populations like immunosuppressed patients. PCT, procalcitonin; IL-6, interleukin-6; SOFA, Sequential Organ Failure Assessment; ICU, intensive care unit; CRP, C-reactive protein.

severe sepsis and septic shock are common complications in patients following an out-of-hospital cardiac arrest and are associated with high mortality. PCT and CRP provide limited diagnostic value for infectious complications, and hence lead to incorrect diagnoses derived from electronic medical records if used as the sole biomarkers [14]. Integrating clinical data with biomarker results leads to improved diagnoses and therapy. Biomarkers should be interpreted dynamically against the background of disease progression, comorbidities, infection risk, imaging, microbiology and vital signs to enable comprehensive decision-making. Unifying interpretations may help standardize use, reduce unnecessary interventions and facilitate early identification and targeted treatment of severe infections.

In addition to diagnosis and risk prediction, biomarker-guided strategies also have practical value in treatment monitoring and antimicrobial stewardship. Evidence-based protocols for combined biomarker assessment in severe infections should define each biomarker's role in diagnosis, monitoring, and treatment evaluation. A PCT-based algorithm guiding antibiotic use in acute pancreatitis reduced unnecessary antimicrobial exposure without compromising safety [15]. Age, comorbidities, and immune status must be considered when setting thresholds and strategies. Overall, future efforts should not only expand biomarker detection but also establish a clinically validated, cost-effective, and dynamically interpretable detection strategy. Through multi-center research, standardized biomarker combinations, thresholds for different disease stages, and clinical decision-making algorithms should be developed. In particular, the detection results of biomarkers should be combined with patients' clinical signs, imaging, microbiology, and electronic health data. Eventually, the combined detection of biomarkers can not only be used for the early diagnosis of infectious diseases, but also for risk stratification, treatment monitoring, and antibiotic management of severe infections.

DECLARATIONS

Author contributions

Lizhou Song and Yunchao Zhou contributed to the manuscript writing and figure preparation; Jibo Zhao designed the work; Lu Yan and Puyong Mi supervised the work. All authors read and approved the final manuscript.

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Data availability

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study. All information is derived from publicly available articles and datasets.

Ethics approval and consent to participate

Not applicable. This manuscript does not contain any studies involving human participants or animals conducted by any of the authors.

Consent for publication

Not applicable. This manuscript does not include details, images, or videos relating to any individual person.

Competing interests

The authors declare that they have no competing interests.

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CASE REPORT

A case report on ultrasound-guided long-axis out-of-plane approach for difficult neuraxial anesthesia

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Abstract

Neuraxial anesthesia is often challenging in patients with obesity, spinal deformity, prior spinal surgery, ankylosing spondylitis, or of advanced age, as conventional landmark-based techniques frequently fail in this population. Ultrasound guidance has improved puncture accuracy; however, commonly used approaches may still be limited by patient positioning and the side from which the operator prefers to work. We report five cases of extremely difficult neuraxial anesthesia in which the procedure was successfully performed using an ultrasound-guided paramedian sagittal long-axis out-of-plane approach. This technique allows continuous visualization of the lamina and the anterior–posterior complex, providing a short and direct puncture path with real-time guidance. All procedures were completed successfully on the first attempt, with short puncture times and no anesthesia-related complications. These cases suggest that the ultrasound-guided long-axis out-of-plane approach is a safe, effective, and flexible option for managing difficult neuraxial anesthesia.

Keywords: Ultrasound guidance, Neuraxial anesthesia, Long-axis out-of-plane approach, Difficult puncture, Case report

1 BACKGROUND

Spinal anesthesia is commonly used for lower extremity, pelvic and lower abdominal surgery. Traditional blind probing relies on well-defined anatomical landmarks; however, in patients with obesity, spinal deformity, history of spinal surgery, ankylosing spondylitis, or degenerative spinal changes, optimal patient positioning for the procedure is often difficult to achieve. This can lead to puncture failure or an increased risk of complications [1-3]. Ultrasound visualization provides real-time image guidance, improving accuracy and safety [4-6]. In ultrasound-guided spinal puncture, the short-axis plane and the paramedian sagittal oblique plane (a long-axis plane) are two common approaches [7-9]. The latter can clearly visualize multiple segments of the lamina, ligamentum flavum, and dural sac (i.e., the "anterior-posterior complex") simultaneously [10]. However, it is more positionally demanding, as right-handed operators typically require the patient to lie on their left side. The long-axis in-plane technique allows direct visualization of the needle trajectory but is often limited by patient positioning and operator handedness (**Figure 1A**). In contrast, the long-axis out-of-plane technique is free from these constraints, allows real-time needle tip tracking, and is ideally suited for complex cases with unclear landmarks, narrow interspaces, or difficult positioning (**Figure 1B**). This paper reports five successful cases of difficult neuraxial anesthesia using the ultrasound-guided long-axis out-of-plane approach.

2 CASE PRESENTATION

2.1 Case 1

A 58-year-old male (120 cm, 35 kg, BMI 24.3 kg/m^2) with congenital chest wall deformity, severe pulmonary dysfunction,

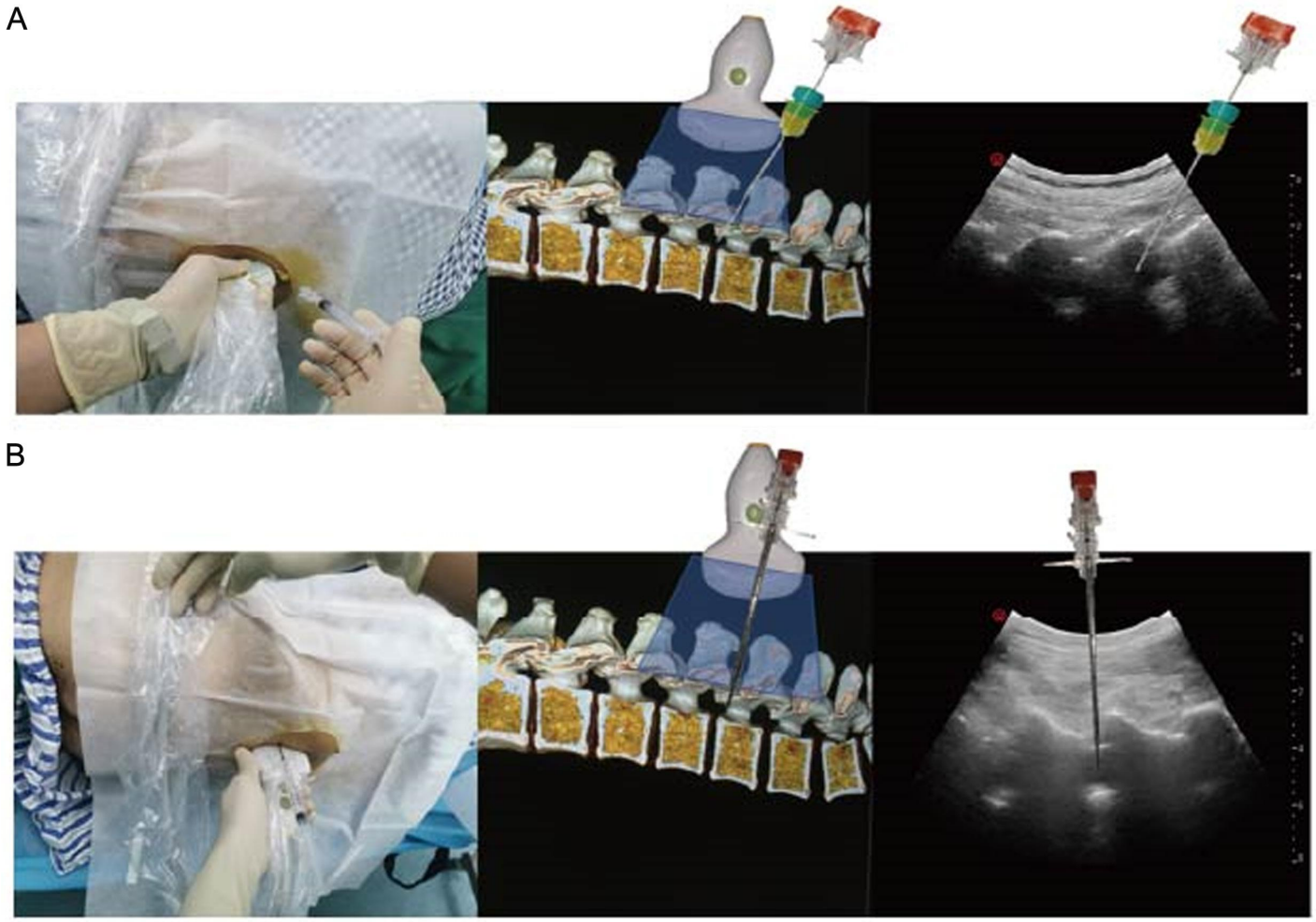

Figure 1. Comparison of long-axis in-plane and out-of-plane ultrasound-guided techniques. Compared with short-axis scanning, the long-axis view provides clearer visualization of intraspinal structures and allows continuous display of multiple interlaminar spaces, facilitating identification of the widest or most distinct acoustic window. The long-axis in-plane technique (A) enables direct visualization of the needle trajectory but is limited by patient positioning and operator handedness. In contrast, the long-axis out-of-plane technique (B) is not constrained by these factors, allows tracking of needle tip advancement, and is particularly suitable for patients with restricted positioning or narrow intervertebral spaces.

and cor pulmonale required transurethral bladder lithotripsy. Spinous processes T6–L2 were impalpable. Ultrasound revealed a clear anterior–posterior complex at L4–L5 and L5–S1. Combined spinal–epidural anesthesia was successfully performed at L4–L5 using a long-axis out-of-plane approach in the left lateral position. Needling time was 5 minutes, achieving a T10 block with stable hemodynamics (**Figure 2A-C**).

2.2 Case 2

A 34-year-old obese parturient (160 cm, 85 kg, BMI 33.2 kg/m^2) at 39 weeks' gestation had a history of lumbar internal fixation and schizophrenia. Previous cesarean section had required general anesthesia. Using laminar long-axis imaging combined with transverse process localization, ultrasound scanning identified the widest anterior–posterior complex at L2–L3. Spinal anesthesia was successfully performed via a long-axis out-of-plane approach within 4 minutes, achieving a T6 sensory level. Doppler ultrasound confirmed intrathecal injection (**Figure 2D-F**).

2.3 Case 3

A 69-year-old patient (158 cm, 57 kg, BMI 22.8 kg/m^2) with prior L4−S1 internal fixation presented with refractory left lumbar radicular pain. Under ultrasound guidance, an epidural puncture was performed at L3–L4 using the long-axis out-of-plane technique, followed by injection of 5 mL of anti-inflammatory and analgesic medication. The patient experienced immediate and significant pain relief (**Figure 2G-I**).

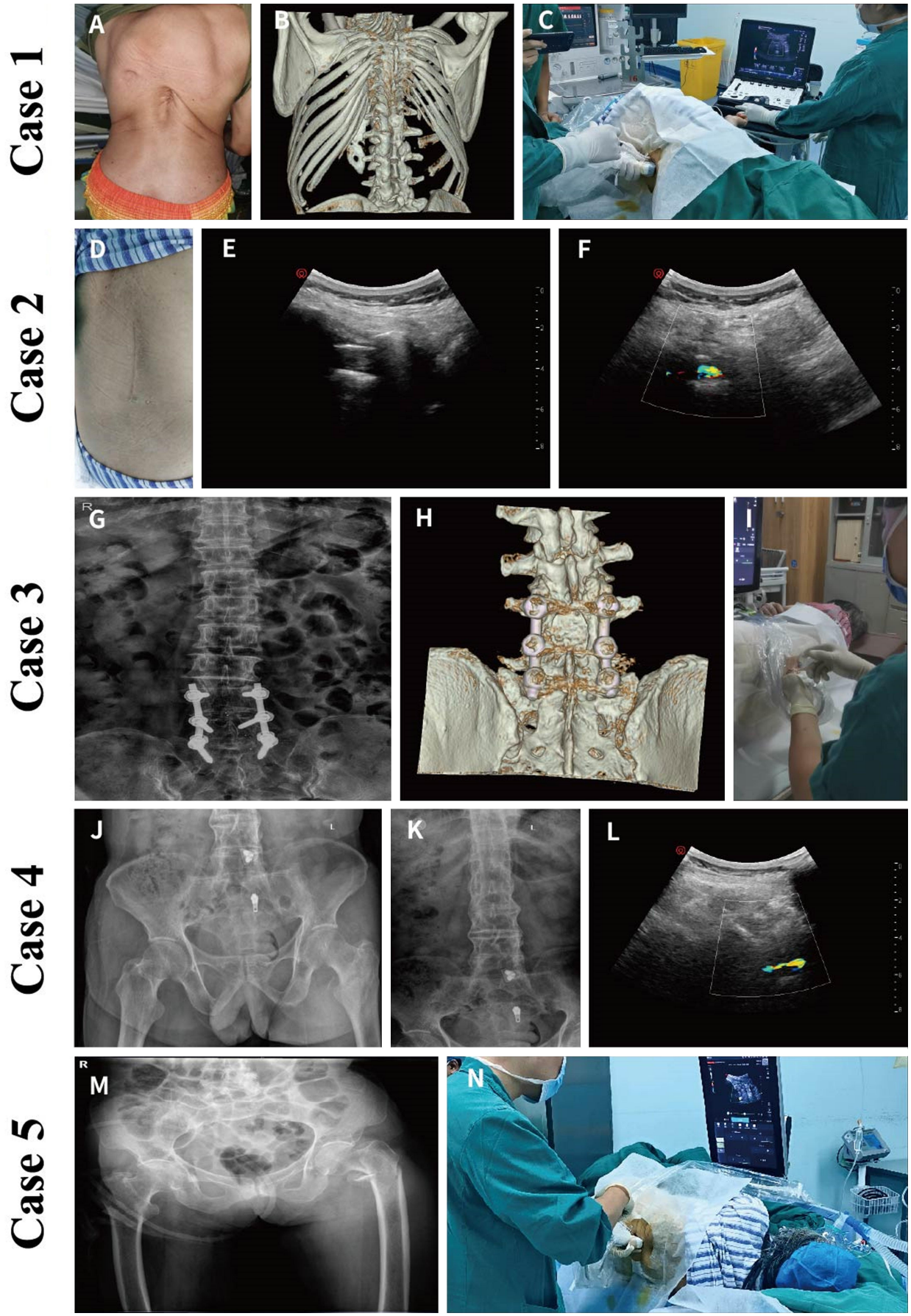
Case 1
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Figure 2. Ultrasound-guided long-axis out-of-plane neuraxial procedures in patients with challenging spinal conditions (Cases 1-5). (A-C) Case 1: A patient with chronic obstructive pulmonary disease and cor pulmonale underwent transurethral cystolithotripsy (A). Three-dimensional reconstruction revealed a congenital thoracic deformity (B). Feasible puncture windows were identified at L4–L5 and L5–S1. With the patient in the left lateral position, combined spinal–epidural anesthesia was successfully performed at L4–L5 using an ultrasound-guided long-axis out-of-plane approach (C). (D-F) Case 2: A parturient with a history of lumbar internal fixation underwent cesarean section (D). Ultrasound scanning demonstrated that the anterior–posterior complex was widest at the L2–L3 interspace (E). Doppler imaging in the short-axis view showed blood flow signals during injectate administration (F). (G-I) Case 3: A patient with a history of L4–S1 lumbar internal fixation presented with left-sided lumbosacral radicular pain (G). Three-dimensional reconstruction demonstrated the internal fixation hardware (H). A puncture window was identified at L3–L4. With the patient in the right lateral position, a diagnostic lateral recess injection was performed at the L3–L4 interspace using an ultrasound-guided long-axis out-of-plane approach (I). (J-L) Case 4: A patient with a left intertrochanteric femoral fracture underwent proximal femoral nail antirotation surgery (J) and had concomitant ankylosing spondylitis (K). With the patient in the right lateral position, spinal anesthesia was performed at the L2–L3 interspace using an ultrasound-guided long-axis out-of-plane approach. Doppler signals generated by injectate spread were observed on the long-axis view (L). (M, N) Case 5: A patient with a left intertrochanteric femoral fracture underwent proximal femoral nail antirotation surgery (M). With the patient in the right lateral position, combined spinal–epidural anesthesia was successfully performed at the L5–S1 interspace using an ultrasound-guided long-axis out-of-plane approach (N).

2.4 Case 4

A 64-year-old male (170 cm, 55 kg, BMI 19.0 kg/m^2) with ankylosing spondylitis and a left intertrochanteric femoral fracture underwent a fascia iliaca compartment block followed by spinal anesthesia. Using ultrasound guidance in the right lateral position, spinal anesthesia was successfully achieved at L2–L3 via a long-axis out-of-plane approach. Doppler signals confirmed injectate spread, and the sensory block reached T8 without complications (**Figure 2J-L**).

2.5 Case 5

A 96-year-old patient (150 cm, 40 kg, BMI 17.8 kg/m^2) with chronic lung disease and a left intertrochanteric fracture posed significant anesthetic challenges. Ultrasound scanning showed that only the L5–S1 anterior–posterior complex was clearly visible. Combined spinal–epidural anesthesia was successfully performed at this level using a long-axis out-of-plane approach. The puncture took 5 minutes, achieved a T10 block, and was well tolerated (**Figure 2M**, **2N**; see Supplementary Video 1).

3 DISCUSSION

This case series includes patients with multiple factors associated with difficult spinal anesthesia, such as spinal deformity, obesity, prior spinal fixation surgery, ankylosing spondylitis, and advanced age. In these populations, conventional landmark-based puncture is often limited by obscure anatomical landmarks, inaccurate localization, and repeated needle attempts [1, 3]. Ultrasound-guided techniques—particularly the long-axis out-of-plane approach—offer an effective solution by enabling direct visualization of the interlaminar space, ligamentum flavum, and dural sac (see Supplementary Video 2) [8, 10]. This approach is not constrained by operator handedness or patient positioning and provides a short, direct puncture path.

The ultrasound-guided long-axis out-of-plane technique has several notable advantages. First, it allows superior visual localization. Compared with short-axis imaging, the long-axis view clearly delineates the spinal canal and enables continuous visualization of multiple interlaminar spaces, facilitating identification of the widest or most distinct acoustic window, especially in patients with absent or distorted surface landmarks [8, 10]. Second, it provides real-time guidance and confirmation. Needle advancement can be dynamically observed as it passes through the ligamentum flavum and dura mater. Correct needle tip placement can be further confirmed by anterior displacement of the dural sac or color Doppler signals generated by saline or drug injection, improving puncture accuracy and safety [9]. Third, this technique aids accurate interspace identification. Laminar oblique scanning combined with transverse process localization can reliably identify the target intervertebral level, which is particularly important in patients with altered spinal anatomy [9, 10]. Finally, in this small series of five patients, the technique was completed successfully on the first attempt in all cases, with short puncture times and no anesthesia-related complications. These preliminary findings suggest the procedure may be feasible and well-tolerated, although further studies with larger sample sizes are needed to evaluate its success rate and safety more definitively.

Several technical considerations are essential for successful application. A low-frequency convex probe is typically used, with paramedian sagittal oblique scanning to align the ultrasound beam parallel to the lamina and obtain an optimal view of the anterior–posterior complex. An out-of-plane needle insertion technique is employed, with needle depth tracked by subtle probe adjustments or the needle trajectory method. In difficult situations, such as when only a single interlaminar space is visible or when severe osteophyte formation is present, probe angulation may need adjustment, and epidural saline injection can assist in confirming loss of resistance and dural sac movement. However, ultrasound penetration is limited in cases of heavy calcification or extensive bony obstruction, underscoring the need for adequate operator training and experience.

4 CONCLUSIONS

In conclusion, this case series of five patients with complex spinal conditions demonstrates that the ultrasound-guided long-axis out-of-plane approach was successfully performed on the first attempt in all cases, with short puncture times and no anesthesia-related complications. These preliminary findings suggest the technique may be a feasible alternative for difficult neuraxial anesthesia, although its efficacy and safety compared with traditional methods cannot be determined from this small series. Further large-scale, controlled studies are warranted to validate these observations and define its potential role in clinical practice.

DECLARATIONS

Author contributions

Liangqing Lin and Qinghua Wu conceptualized the study and designed the methodology. Pinhui Ke and Chunlan Lin were responsible for the data curation and formal analysis. Yaohua Yu performed the investigation and validated the results. All authors contributed to writing the original draft, reviewing, and editing the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data availability

The individual deidentified participant data can be accessed from the corresponding author (Yaohua Yu, yyh.8@163.com) upon reasonable request.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of The First Hospital of Putian (Approval No. 2026-026), and written informed consent was obtained from all participants or their legal representatives.

Consent for publication

All the authors have read and approved the final version of the manuscript and have consented to its submission for publication. The manuscript is not under consideration for publication elsewhere.

Competing interests

The authors declare that they have no competing interests.

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Supplementary Information

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Perioperative Precision Medicine

Zentime

PERSPECTIVE

Critical thresholds in perioperative blood pressure management: A precision strategy for preventing postoperative organ complications

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1 INTRODUCTION

Perioperative blood pressure (BP) control is a cornerstone of anesthesiology, as it significantly affects organ recovery and the development of postoperative complications. In pediatric patients undergoing cardiopulmonary bypass, brain injury is associated with impaired cerebral autoregulation. Accurate adjustment of mean arterial pressure (MAP) can preserve cerebral perfusion and thus minimize neurological injury [1]. Hypotension often leads to insufficient perfusion of the brain, heart, and kidneys, resulting in complications such as acute kidney injury (AKI) and cerebral ischemia.

Recent advancements have shifted perioperative BP management from empirical to goal-directed approaches. For instance, transcranial Doppler monitors cerebral blood flow following vascular occlusion to prevent hyperperfusion. Tools such as esophageal Doppler and the pressure recording analytical method (PRAM) enable more precise titration of circulatory dynamics. Low-dose continuous norepinephrine (NE) infusion combined with goal-directed fluid therapy has been shown to reduce complications in elective pulmonary surgery [2]. The prophylactic use of vasoactive agents and the renal-protective effects of dexmedetomidine (DEX) provide additional strategies for effective perioperative BP control. Defining personalized safety thresholds and integrating multimodal monitoring remain critical challenges. Notably, lower MAP is strongly associated with postoperative AKI, while post-induction hypotension independently increases the odds of adverse events in transcatheter aortic valve replacement [3].

2 MECHANISMS AND IMPACT OF PERIOPERATIVE BP MANAGEMENT

2.1 Effects of BP fluctuations on organ perfusion

Hemodynamic changes during the perioperative period can exert a significant impact on organ perfusion and function. In patients with aneurysmal subarachnoid hemorrhage, the oxygen reactivity index shows superior sensitivity in detecting perfusion derangements associated with delayed cerebral ischemia [4]. While compensatory microcirculatory mechanisms may preserve certain mucosal functions during hypotension, accurate BP control remains vital for critical organs. Personalized BP control based on dynamic hemodynamic variations may attenuate the risk of perfusion mismatch that leads to organ injury. Current clinical consensus suggests that maintaining MAP within 10% of the patient's baseline or keeping the cerebral oxygenation index below 0.3 serves as a critical threshold for organ protection.

2.2 Correlation between hypotension and postoperative cerebral complications

Perioperative hypotension contributes to cerebral complications through reduced perfusion pressure and disruption of the

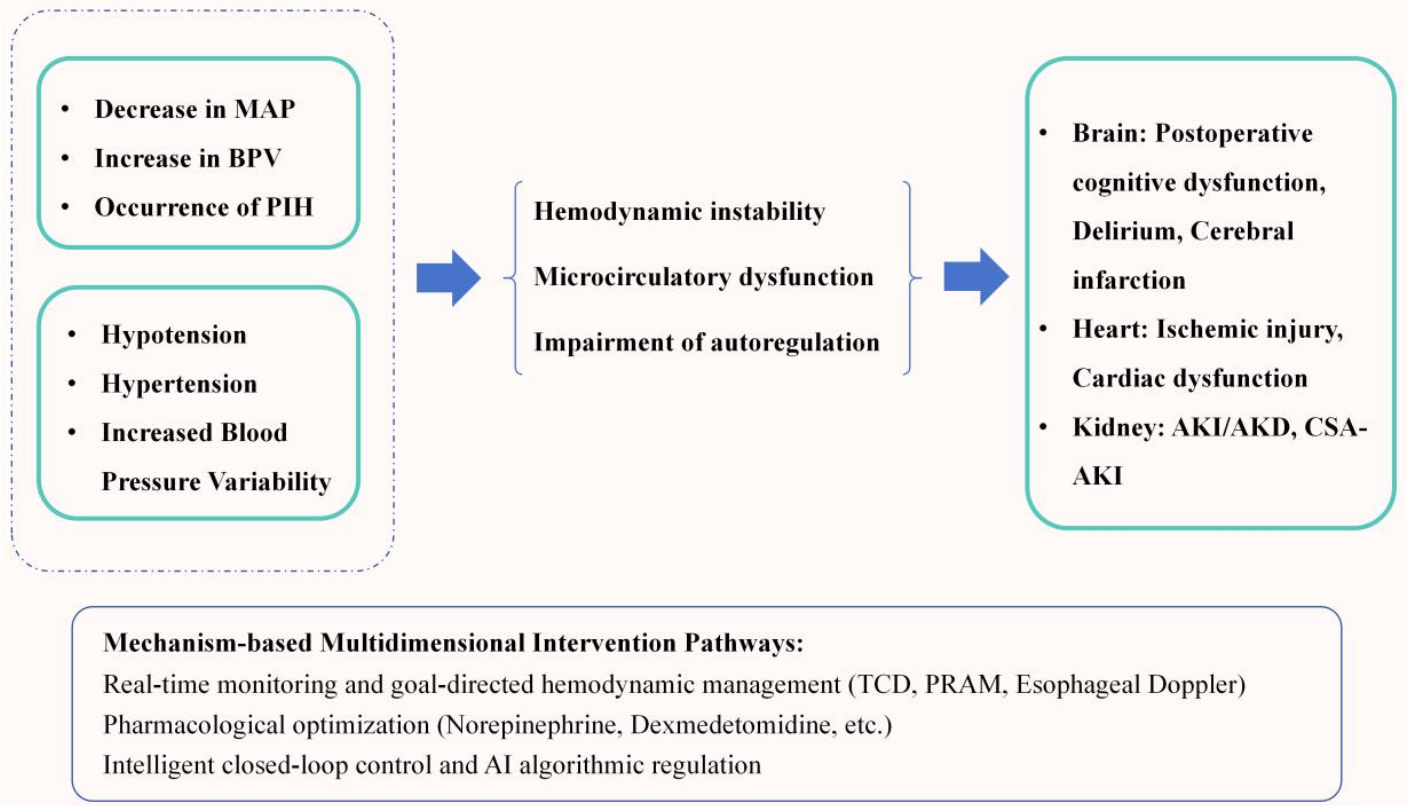

Figure 1. Schematic representation of critical thresholds in perioperative blood pressure management and multi-organ protection strategies. Decreases in MAP, increases in BPV, and PIH lead to hemodynamic instability, microcirculatory disturbances, and impaired autoregulation, resulting in subsequent injury to critical organs such as the brain, heart, and kidneys. Mechanism-based multidimensional intervention strategies include dynamic monitoring and goal-directed hemodynamic therapy (e.g., TCD, PRAM, esophageal Doppler), pharmacological optimization (e.g., norepinephrine, dexmedetomidine), and closed-loop control using artificial intelligence for real-time BP management. This figure was created by the author using Figdraw. MAP, mean arterial pressure; BPV, blood pressure variability; PIH, post-induction hypotension; TCD, transcranial Doppler; PRAM, pressure recording analytical method; BP, blood pressure; AKI, acute kidney injury; AKD, acute kidney disease; CSA-AKI, cardiac surgery-associated acute kidney injury; AI, artificial intelligence.

blood–brain barrier. Orthostatic hypotension is associated with cognitive impairment and microstructural brain damage. However, personalized protocols that maintain cerebral oxygenation within optimal ranges show promise for reducing neurological complications. In addition, acute water intake has been shown to improve orthostatic tolerance in patients with orthostatic hypotension and reduce performance decline associated with insufficient cerebral perfusion, offering a simple perioperative neuroprotective strategy [5]. A critical threshold is typically defined as an absolute MAP below 65 mmHg or a decrease of more than 20% from the pre-induction baseline, both of which significantly elevate the risk of cognitive impairment.

2.3 BP-related mechanisms of cardiac and renal dysfunction

Perioperative BP variability profoundly impacts hemodynamic stability and organ perfusion, thereby influencing both cardiac and renal function. Intraoperative hypotension—especially a decrease in MAP—can lead to insufficient myocardial perfusion and ischemic damage; therefore, stable BP is essential for cardiac protection. Higher blood pressure variability (BPV) during cardiopulmonary bypass—particularly when MAP fluctuations exceed 30% of the area under the curve—is significantly associated with cardiac surgery-associated AKI in pediatric patients [6]. For adults, a cumulative duration of MAP below 60 mmHg for more than 20 minutes is identified as a critical threshold for stage 1 AKI.

Perioperative hemodynamic instability—characterized by chronic or repeated episodes of hypotension, hypertension, or increased BPV—leads to microcirculatory deterioration and disruption of autoregulatory responses, ultimately resulting in target organ injury (**Figure 1**). Notably, patients with chronic hypertension are more prone to these variations due to preexisting arteriolosclerosis and impaired renal autoregulation. This cardiorenal interaction illustrates the complex relationship between hemodynamic instability and organ perfusion in a vulnerable state. Such pathophysiological processes highlight the importance of personalized and dynamic BP management to reduce intraoperative shock and improve early postoperative outcomes.

3 INNOVATIVE MANAGEMENT STRATEGIES AND CLINICAL PRACTICE

Individualized BP management guided by real-time monitoring is essential for preventing complications. Esophageal Doppler-guided therapy decreases pulmonary complications and hospital length of stay, while the PRAM facilitates postoperative triage and reduces healthcare costs. Continuous arterial pressure monitoring and AI-based algorithms enable clinicians to dynamically assess circulatory status. Closed-loop control systems further improve hemodynamic stability. Accurate pharmacological regulation effectively protects organ function. Prophylactic NE infusion is superior to bolus epinephrine for reducing post-induction hypotension and complications in major abdominal surgery. Continuous DEX infusion for 24 hours significantly lowers the rate of AKI rates by 29% without hemodynamic risks. Titratable, short-acting α-adrenergic agonists permit stable perfusion and minimize organ injury. Furthermore, closed-loop administration of NE significantly decreases the incidence of postoperative hypotension in ICU patients following cardiac surgery [7].

The integration of continuous BP monitoring with advanced AI algorithms facilitates real-time recognition of hemodynamic changes. Notably, the ClearSight system provides safe, non-invasive arterial pressure measurement via a finger cuff, offering a viable alternative for neurovascular surgery. In the future, these intelligent technologies could be incorporated into a "prediction–prevention–intervention" model to provide more precise control and further reduce perioperative complications [8]. A variety of commonly used perioperative antihypertensive drugs with differentiated administration regimens and corresponding clinical trial outcomes are summarized in Supplementary Table 1.

4 DISCUSSION

4.1 Multidisciplinary collaboration in perioperative BP management

Optimization of perioperative BP is an important factor in preventing postoperative organ dysfunction, and collaboration among various disciplines (anesthesiology, surgery, critical care, and nursing) is crucial for its successful implementation. Real-time monitoring-guided, patient-specific BP management strategies may facilitate optimal intraoperative and postoperative decision-making. For instance, intraoperative transcranial Doppler monitoring allows early detection of cerebral hyperperfusion syndrome after carotid endarterectomy, whereas esophageal Doppler and PRAM improve the reliability of hemodynamic data and contribute to continuity of care [9]. With multidisciplinary support, encephaloduroarteriosynangiosis can be optimally performed with further improvements in surgical techniques, strict hemodynamic control during anesthesia, and versatile methods of medical management [10]. In the future, establishing standardized collaborative workflows, improving information exchange, and enhancing team training will be crucial for transitioning BP intervention from isolated treatment to coordinated, whole-process management.

4.2 Key challenges and emerging research priorities

Despite exciting advances in personalized BP management for preventing postoperative organ injury, major hurdles remain in implementing this approach at the bedside. The use of real-time monitoring systems is restricted by their availability and the lack of standardized operating procedures, which hinders their application in primary hospitals. BPV can influence cerebral perfusion and is associated with the development of ICU delirium; however, its mechanistic involvement in organ injury, diagnostic parameters, and therapeutic targets have yet to be elucidated [11]. Combined pharmacological and technological approaches, such as closed-loop NE infusion systems, appear promising for improving BP control; however, there is a lack of evidence regarding their long-term beneficial impact and cost-effectiveness. Furthermore, the ClearSight system enables non-invasive arterial pressure measurement via a finger cuff and may be a safe alternative for use in neurovascular surgery [8]. Future research should focus on intelligent, non-invasive monitoring technologies, multicenter randomized controlled trials, and integrated models that combine biomarkers with hemodynamic parameters to enable precise risk stratification and targeted interventions.

4.3 The role of BP management in overall postoperative recovery

Precise perioperative BP management can markedly lower the incidence of complications in vital organs such as the brain, heart, and kidneys and improve microcirculation and postoperative lung function. These factors contribute to reduced hospital length of stay and improved long-term outcomes. In cardiac surgery, intraoperative DEX can preserve renal function and reduce the use of vasopressors [12]. Goal-directed hemodynamic therapy makes pulmonary recovery more efficient and improves overall rehabilitation. Additionally, combined aerobic and resistance training has been shown to be more effective than aerobic training alone in reducing BP and its variability [13]. Integrating BP management into a comprehensive "surgery–monitoring–rehabilitation" pathway, together with early mobilization and nutritional support, enables the establishment of a patient-centered, multidimensional rehabilitation system that substantially improves the overall quality of perioperative care.

5 CONCLUSION AND CLINICAL IMPLICATIONS

Accurate perioperative BP control is essential for avoiding postoperative multiorgan failure. Although current evidence highlights the critical importance of personalized manage-

ment, several clinical gaps remain. One major limitation is the lack of universal, high-level evidence that specifically defines "critical thresholds" across diverse surgical populations. Most current studies rely on retrospective data, which may not fully account for individual patient baseline variability [14].

Future research should prioritize multicenter randomized controlled trials to validate the long-term cost-effectiveness and clinical outcomes of intelligent closed-loop infusion systems. Furthermore, integrating non-invasive monitoring technologies with machine-learning algorithms will be crucial for transitioning from reactive treatment to proactive, predictive interventions.

Finally, the successful implementation of these strategies requires robust multidisciplinary collaboration and standardized educational protocols. The ultimate goal is to establish a patient-centered, multidimensional "prediction–prevention–rehabilitation" pathway that ensures safer perioperative recovery, even in resource-limited settings.

ABBREVIATIONS

MAP, mean arterial pressure; BP, blood pressure; DEX, dexmedetomidine; AI, artificial intelligence; NE, norepinephrine; ICU, intensive care unit; PRAM, pressure recording analytical method; AKI, acute kidney injury; BPV, blood pressure variability.

DECLARATIONS

Author contributions

Yanxi Liu was responsible for the initial conceptualization and design of this article, conducted a comprehensive literature search and content extraction, performed a critical analysis of perioperative blood pressure management strategies, and drafted the original manuscript. The author also undertook the subsequent revisions and final proofreading, and approved the submitted version for publication.

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Data availability

Data sharing is not applicable to this article, as no new datasets were generated or analyzed during the current study.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The author declares that there are no competing interests regarding the publication of this paper.

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Supplementary Information

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Supplementary Information

Supplementary Table 1. Key clinical research elements in intraoperative blood pressure pharmacologic regulation strategies

Intervention drug	Administration strategy	Patient population/ type of surgery	Target blood pressure management scenario	Secondary outcomes	Primary outcomes	Reference
Vasopressors such as metaraminol	High intraoperative dosage	Elderly patients undergoing non-cardiac surgery	Mean arterial pressure (MAP) <60, systolic blood pressure (SBP) <90	Decrease in hemoglobin levels	Postoperative AKI	[1]
Esketamine	Continuous infusion at 0.25 mg/kg/h	Patients undergoing below-knee orthopedic surgery	Blood pressure regulation during tourniquet inflation	Intraoperative hypotension and postoperative adverse reactions	Incidence of tourniquet-induced hypertension	[2]
Magnesium sulfate	Intraoperative intravenous infusion	Postoperative patients undergoing abdominoplasty after weight loss	Management of systolic blood pressure during tracheal intubation and skin incision	Ephedrine and propofol consumption and pain scores	Changes in systolic blood pressure during tracheal intubation and skin incision	[3]
Propofol versus sevoflurane	Intravenous infusion versus inhalation	Patients undergoing abdominal laparoscopic surgery	Maintenance of MAP with phenylephrine	Postoperative pain and opioid consumption	Changes in nociception level (NOL) index after tetanic stimulation	[4]
Sufentanil combined with propofol	Total intravenous anesthesia with combined administration	Patients undergoing radical mastectomy	Stable intraoperative blood pressure and heart rate (HR)	Stress indicators, pain scores, and adverse reactions	Hemodynamic parameters remain stable	[5]
Ketamine, dexmedetomidine	Intravenous bolus followed by continuous infusion	American society of anesthesiologists physical status (ASA) I/II patients undergoing laparoscopic cholecystectomy	Maintains intraoperative hemodynamic stability	Comparable recovery quality and side effects	Reduced anesthetic and analgesic consumption	[6]
Dexmedetomidine	(1 μg/kg) as an adjuvant to ropivacaine	Pediatric upper limb fracture surgery	Comparison of intraoperative blood pressure and HR	Rescue analgesia and complications	Prolonged duration of analgesia maintenance	[7]
Dexmedetomidine	Intravenous administration before anesthesia induction	Patients undergoing laparoscopic cholecystectomy	Maintains hemodynamic stability	Reduces incidence of coughing	Hemodynamic stability	[8]
Phenoxybenzamine (PXB)	Duration of preoperative PXB administration	Patients undergoing pheochromocytoma and paraganglioma (PPGL) resection	Intraoperative hemodynamic targets	Hypotension, infection	Hemodynamic instability	[9]
Dexmedetomidine	Continuous infusion at 0.5-1.0 μg/kg/hour	Laparoscopic colorectal cancer surgery	Intraoperative blood pressure measurement at multiple time points	MAP, HR, and bispectral index (BIS)	Reduced postoperative pain scores	[10]
Dexmedetomidine	Intravenous injection of 0.5 μg/kg before anesthesia induction	ASA I/II grade patients undergoing laparoscopic cholecystectomy (LC)	Perioperative blood pressure management during general anesthesia for LC	Anesthetic consumption, recovery time, and adverse events	Hemodynamic stability and attenuation of stress response	[11]
Ketamine, magnesium sulfate	Adjuvant for scalp block	Patients undergoing supratentorial craniotomy	Blood pressure control during pin insertion	Changes in MAP and HR	Reduction in postoperative pain scores	[12]
Hyoscine butyl bromide	Prophylactic intravenous injection of 20 mg	Spinal anesthesia for cesarean section in parturients	Prevention of intraoperative bradycardia	Reduction in the incidence of nausea and vomiting	Decrease in the incidence of bradycardia	[13]

Esmolol	Single dose of 0.5-1.0 mg/kg before induction, continuous infusion of 0.5-2.0 mg/kg/h during surgery	ASA II/III surgical patients	Perioperative intubation and extubation responses	Intraoperative and postoperative opioid consumption and adverse events	Incidence of intubation and extubation responses	[14]
Salbutamol/budesonide	Intraoperative inhalation administration	Elderly patients with high cardiovascular risk undergoing thoracoscopic lobectomy	Intraoperative blood pressure maintained within 20% of baseline	Reduced postoperative pulmonary complication score	Decreased incidence of myocardial injury after non-cardiac surgery (MINS)	[15]

Note: AKI, acute kidney injury; ASA, American society of anesthesiologists physical status; BIS, bispectral index; HR, heart rate; LC, laparoscopic cholecystectomy; MAP, mean arterial pressure; MINS, myocardial injury after non-cardiac surgery; NOL, nociception level; PPGL, pheochromocytoma and paraganglioma; PXB, phenoxybenzamine; SBP, systolic blood pressure.

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Perioperative Precision Medicine

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PERSPECTIVE

The dual nature of oxidative stress: DNA damage and immune activation

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Oxidative stress serves as the central pathological hub of various major diseases such as cancer, neurodegenerative disorders, sepsis, and perioperative organ injury. Its core is an imbalance between reactive oxygen species (ROS) production and the body's antioxidant defense system, which can bidirectionally regulate DNA damage and immune activation. Therefore, understanding its homeostatic mechanisms is critical for disease prevention and treatment, particularly in organ protection and prognosis improvement for perioperative anesthetized and critically ill patients (**Figure 1**).

1 THE IMBALANCED ESSENCE OF OXIDATIVE STRESS

The core of oxidative stress is the functional mismatch between ROS generation including superoxide anion, hydrogen peroxide and other species, and the activity of antioxidant systems including superoxide dismutase, glutathione and catalase, rather than an absolute increase in ROS levels. Its biological effects are highly dependent on concentration, duration of action and cellular compensatory capacity [1].

Moderate, controlled oxidative stress can function as a physiological signaling cue to trigger immune responses and facilitate pathogen clearance; conversely, excessive, sustained oxidative stress induces irreversible damage to biological macromolecules including lipids, proteins and DNA [1]. Taking the classic oxidative stress inducer H_2O_2 as an example, at low concentrations ranging from nanomolar to low micromolar levels, ROS govern key signaling pathways such as protein kinase B phosphorylation via reversible redox modification; once concentrations exceed the cellular tolerance threshold such as ≥100 μM, ROS shift to a potent cytotoxic agent that triggers cell death and tissue injury [2]. Notably, telomeric DNA at chromosome ends is highly vulnerable to ROS attack. Persistent oxidative stress accelerates telomere shortening and dysfunction, induces cellular senescence and genomic instability, features that are also core mechanisms underlying inflammatory diseases, malignant tumor progression, and accelerated perioperative organ senescence [2]. Clinical scenarios including perioperative ischemia-reperfusion, mechanical ventilation, anesthetic exposure and sepsis are all key triggers of systemic oxidative stress imbalance in the body.

2 DNA DAMAGE EFFECTS MEDIATED BY OXIDATIVE STRESS

Sustained ROS attack on DNA elicits multiple forms of damage, including base oxidation typified by 8-oxoguanine formation, DNA strand breaks, and protein-DNA cross-linking. The body executes damage repair via multi-tiered mechanisms such as base excision repair, nucleotide excision repair and homologous recombination repair. Among these, key base excision repair pathway enzymes such as 8-oxoguanine DNA glycosyl-

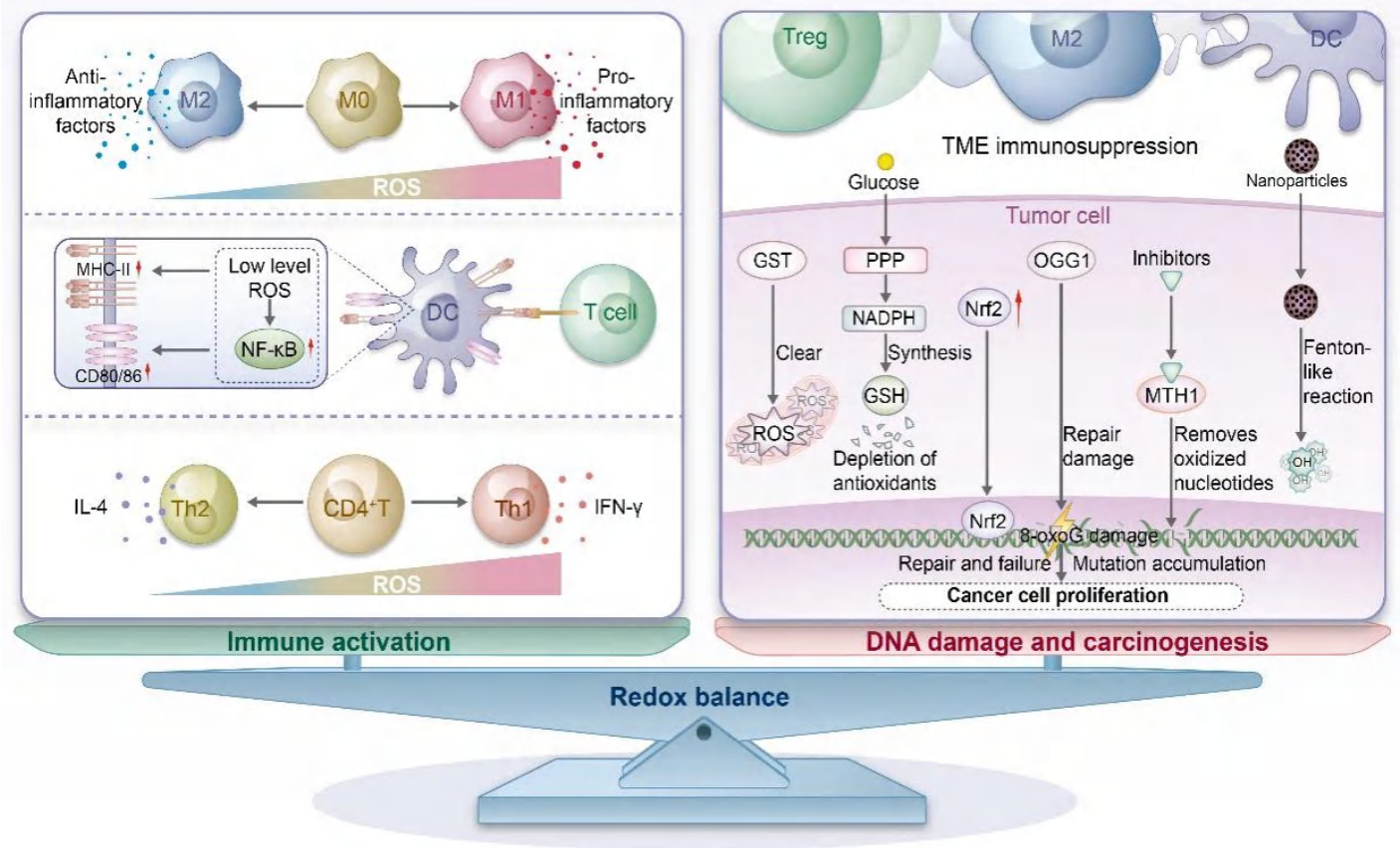

Figure 1. The dual roles of oxidative stress: balance, imbalance, and targeted intervention. ROS serve as critical signaling molecules that regulate immune activation. They promote macrophage polarization, enhance dendritic cell antigen presentation via activation of the NF-κB pathway, and regulate $CD4^+$ T cell differentiation to maintain immune surveillance. Excess ROS can directly induce DNA damage in cells. Although OGG1 is involved in repairing such damage, sustained ROS exposure results in uncontrolled DNA lesions and increased genomic instability. Cancer cells maintain relatively high ROS levels to activate proliferative signaling pathways, and they enhance their antioxidant capacity via Nrf2 upregulation and metabolic reprogramming to drive tumor progression. Multiple targeted intervention strategies have been developed for oxidative stress dysregulation. Nanocatalytic materials can disrupt the redox balance of tumor cells and induce DNA damage via localized Fenton-like generation of hydroxyl radicals, GSH depletion, and blockade of DNA repair pathways. ROS, reactive oxygen species; NF-κB, nuclear factor kappa B; $CD4^+$ T cell, cluster of differentiation 4-positive T cell; OGG1, 8-oxoguanine DNA glycosylase 1; Nrf2, nuclear factor erythroid 2–related factor 2; GSH, glutathione; M0, M0 macrophage; M1, M1 macrophage; M2, M2 macrophage; DC, dendritic cell; Treg, regulatory T cell; Th1, T helper 1 cell; Th2, T helper 2 cell; IL-4, interleukin-4; IFN-γ, interferon-gamma; MHC-II, major histocompatibility complex class II; CD80/86, cluster of differentiation 80/86; TME, tumor microenvironment; GST, glutathione S-transferase; PPP, pentose phosphate pathway; NADPH, nicotinamide adenine dinucleotide phosphate; MTH1, MutT homolog 1; 8-oxoG, 8-oxoguanine.

ase 1 not only directly repair oxidized bases, but also preserve tissue barrier integrity and immune microenvironment homeostasis by regulating cytokine expression [3].

The primary pathogenic consequence of persistent oxidative stress is not mere cytotoxicity, but the accumulation of oncogenic mutations driven by induced genomic instability, coupled with epigenetic alterations including DNA methylation and histone modification. These changes collectively drive uncontrolled cell proliferation and apoptotic evasion, ultimately promoting tumor initiation and progression [1]. Cancer cells consistently maintain higher ROS levels than normal cells: on one hand, they drive proliferative and survival signaling via activation of pathways such as mitogen-activated protein kinase-extracellular signal-regulated kinase and phosphoinositide 3-kinase-protein kinase B; on the other hand, they counteract the cytotoxicity of elevated ROS by upregulating antioxidant transcription factors such as nuclear factor erythroid 2-related factor 2 (Nrf2) and reprogramming metabolic pathways, thereby sustaining malignant progression [4]. In the fields of anesthesiology and critical care medicine, persistent oxidative stress induced by pathological states including perioperative ischemia-reperfusion, cardiopulmonary bypass, and sepsis disrupts DNA damage-repair homeostasis in vital organ cells such as renal tubular epithelial cells, alveolar epithelial cells, and cardiomyocytes. This disruption represents the core pathological mechanism underlying the development of postoperative multiple organ dysfunction syndrome.

3 OXIDATIVE STRESS AND IMMUNE ACTIVATION

Oxidative stress exhibits a tightly regulated threshold effect in modulating immune responses. Physiological levels of ROS are indispensable for normal immune system activation: during the innate immune phase, ROS promote macrophage polarization toward the pro-inflammatory M1 phenotype, stimulate the release of cytokines such as tumor necrosis factor-alpha and interleukin-1 beta, and enhance Toll-like receptor pathway activity and macrophage bactericidal capacity [5]. During antigen presentation, ROS upregulate dendritic cell expression of major histocompatibility complex class II molecules and co-stimulatory molecules via nuclear factor kappa-B pathway activation, boosting antigen presentation efficiency [6]. During the adaptive immune phase, ROS finely tune T cell activation and differentiation to maintain normal host defense function [7].

However, when oxidative stress remains chronically uncontrolled, physiological immune activation quickly transitions into chronic inflammation and immune dysfunction, acting as a key pathogenic driver of disease progression. This effect is particularly pronounced in the tumor microenvironment: ROS produced by cancer cells and stromal cells induce the infiltration of immunosuppressive cells, establishing a self-reinforcing positive feedback loop between oxidative stress and inflammation that ultimately drives immune escape [8]. In perioperative and critical care settings, this threshold effect directly determines patient prognosis: uncontrolled oxidative stress in patients with sepsis and acute respiratory distress syndrome not only triggers cytokine storms to exacerbate tissue injury, but also induces aberrant macrophage polarization and T cell exhaustion, leading to immune paralysis and significantly increasing the risk of secondary infection and mortality. Meanwhile, anesthetic agents and perioperative management strategies can modulate postoperative immune function and infection risk by regulating systemic oxidative stress levels.

4 PRECISE INTERVENTION STRATEGIES BASED ON REDOX HOMEOSTASIS

The dual nature of oxidative stress dictates that the core of clinical intervention is precision-targeted homeostasis control—excessive oxidative stress can be corrected via antioxidant strategies to mitigate DNA damage for disease prevention and organ protection, while moderate oxidative stress can be strategically harnessed in specific contexts to activate immunity, or tumor cell redox homeostasis can be selectively disrupted to induce cancer cell death.

Nevertheless, traditional non-selective antioxidant therapies carry marked clinical limitations. Multiple large-scale clinical trials and basic studies have confirmed that universal antioxidant supplements yield no preventive or therapeutic benefits, and instead increase lung cancer risk in smokers and accelerate tumor growth in murine models, fully demonstrating that disruption of redox homeostasis on either end exacerbates pathological progression [9, 10]. In anesthesiology and critical care medicine, regulating redox balance has emerged as a research focus for perioperative organ protection and critical care management. Use of volatile anesthetics and clinically routine agents such as dexmedetomidine can moderately modulate ROS levels and activate the Nrf2 antioxidant pathway, attenuating ischemia-reperfusion induced DNA damage and immune dysfunction to confer perioperative protection for vital organs including the heart, brain and kidneys. For sepsis treatment, research has abandoned non-selective antioxidant therapy, shifting focus to precise intervention strategies that target excess ROS clearance while preserving the physiological immune-activating function of ROS.

In addition, strategies targeting the disruption of tumor cell redox homeostasis have demonstrated notable translational potential. For instance, nanocatalytic platforms can specifically amplify oxidative damage in tumor tissues and block DNA repair pathways to achieve precision anti-tumor therapy. The development of detection technologies for highly sensitive oxidative stress biomarkers such as 8-oxo-7,8-dihydro-2'-deoxyguanosine has also laid critical groundwork for accurate assessment of patient oxidative stress status and implementation of individualized interventions [11].

5 CONCLUSION

In summary, oxidative stress modulates two core biological processes, DNA damage and immune activation, simultaneously via concentration-dependent bidirectional effects. Future research and clinical practice should focus on the precise identification and control of redox balance points in specific pathological microenvironments, minimizing pathological DNA damage while preserving or enhancing physiological immune activation, ultimately delivering optimized strategies for the prevention and treatment of tumors, perioperative organ injury, severe sepsis and other related diseases.

DECLARATIONS

Author contributions

Yalin Zhu and Long Peng contributed to the manuscript writing and figure preparation, Yalin Zhu and Wen Xu designed the work, Yimin Zhang, Haiwen Wang, and Wen Xu supervised the study. All authors have read and approved the article.

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Not applicable.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable. This manuscript does not include details, images, or videos relating to an individual person.

Competing interests

The authors declare that they have no conflict of interest.

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Perioperative Precision Medicine

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RESEARCH ARTICLE

Acupoint selection patterns and clinical evidence for acupuncture in the management of breast cancer-related pain

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Abstract

Objectives: This study aimed to identify acupoint selection patterns for breast cancer-related pain using the Knowledge Discovery in Databases (KDD) method, construct a data-informed candidate acupoint prescription, and preliminarily evaluate its clinical efficacy when combined with the World Health Organization three-step analgesic ladder. **Methods:** Databases including China National Knowledge Infrastructure, VIP Information, WanFang, PubMed, and Web of Science were searched from inception to May 2024. An Excel-based dataset was developed to analyze acupoint frequency and meridian attribution. Acupuncture prescriptions for cancer pain without specifying cancer type were retained as potentially informative references for breast cancer-related pain. Sixty eligible patients were randomized into two groups. The control group received standard three-step analgesics, including celecoxib, tramadol sustained-release, or oxycodone sustained-release according to pain severity. The intervention group received the same regimen plus a KDD-derived acupuncture prescription consisting of bilateral Hegu (LI4), Zusanli (ST36), Neiguan (PC6), Taichong (LR3), Sanyinjiao (SP6), and Ashi points. Needles were retained for 30 minutes using balanced stimulation, five sessions per week for three weeks. Outcomes included pain intensity, breakthrough pain frequency, duration of pain relief, Karnofsky Performance Status, sleep quality, depressive symptoms, analgesic efficacy, quality of life, and safety. **Results:** The KDD-derived candidate prescription was associated with a higher overall response rate than analgesics alone. Patients receiving adjunctive acupuncture showed greater reductions in pain intensity and breakthrough pain episodes, as well as improvements in functional status, sleep quality, and depressive symptoms. Fewer adverse events were reported in the acupuncture group. **Conclusions:** This study proposes a potential acupoint combination for relieving breast cancer-related pain based on distal meridian points and local tender points. Combined acupuncture and conventional pharmacotherapy may improve analgesic outcomes, quality of life, and psychological status, with a favorable safety profile.

Keywords: Acupuncture, Breast cancer, Cancer-related pain, Acupoint selection patterns, Clinical efficacy

1 INTRODUCTION

Breast cancer accounts for approximately one-quarter of all female cancers and is responsible for approximately 15% of cancer-related deaths among women worldwide [1, 2]. The global burden of the disease is unevenly distributed: high-income countries often exhibit a pattern of higher incidence but lower mortality, whereas low- and middle-income regions

demonstrate the opposite trend [3, 4]. In China, both the incidence and mortality of breast cancer continue to rise, with the highest incidence observed in women aged 45–59 years [5]. Although the implementation of widespread screening and treatment has led to national improvements, with a 5-year survival rate exceeding 70%, less developed regions report survival rates of only approximately 60%, indicating persistent geographic disparities [6]. A substantial proportion of patients with breast cancer experience cancer-related pain, which seriously impairs quality of life (QoL) and therapeutic outcomes, particularly in advanced stages of the disease.

Breast cancer may present as a painless breast lump or with more advanced features, including skin alterations, nipple discharge and retraction, local pain, and axillary lymphadenopathy [2]. The etiology of breast cancer is complex and depends on a combination of factors, including estrogen exposure, genetic predisposition, environmental factors, body constitution type, reproductive history, as well as socioeconomic and psychosocial issues [7, 8]. According to the International Association for the Study of Pain, pain is defined as an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage [9]. Pain is among the most common and disabling symptoms in oncology [10, 11]. When poorly managed, pain has a far-reaching impact on physical fitness, emotional well-being, and cognitive function, frequently precipitating anxiety, depression, and significant deterioration in QoL [12, 13]. The sources of breast cancer-related pain can be grouped into five domains: (1) Tumor-related pain: resulting from invasion or direct compression of surrounding tissues, nerves, and vasculature by the primary tumor, or from subclinical inflammatory responses elicited by tumors. (2) Bone metastasis: bone is one of the most common distant metastatic sites in advanced breast cancer, with involvement reported in approximately 65–75% of patients with metastatic disease [14, 15]. (3) Treatment-related pain: arising from surgical trauma, fibrosis or neuropathies caused by radiotherapy, and tissue or nerve injury related to chemotherapy. (4) Pain due to other chronic conditions. (5) Psychosocial aspects: including anticipation of death, surgical trauma, disability, and economic stress; all of these factors increase the perception and intensity of pain.

Tumor-related pain frequently occurs in the context of active treatment and progressive disease. In 1982, the World Health Organization (WHO) established the WHO three-step analgesic ladder, which serves as a guide for treating cancer pain [16]. Optimal pain control usually employs a multimodal strategy incorporating both pharmacological and non-pharmacological components. Pharmacotherapy may consist of non-opioid analgesics (including paracetamol or NSAIDs), adjuvant agents (such as antidepressants, anticonvulsants, topical formulations, and corticosteroids), and, when appropriate, opioid analgesics [17]. For opioid-naïve patients with mild to moderate pain, weak opioids such as codeine, hydrocodone, or tramadol are recommended, with no meaningful differences in analgesic efficacy reported among these agents [18, 19]. These agents are often used in combination with paracetamol or other non-opioid analgesics, including NSAIDs. When pain becomes moderate to severe, strong opioids like morphine, oxycodone or hydromorphone are recommended. Management should start with low doses and titration to an optimum between satisfactory analgesia and acceptable side effects [20]. The evolution of neuropathic pain classification has contributed to a paradigm shift in symptom management. In modern oncology, the WHO three-step analgesic ladder, including NSAIDs, opioids, and adjuvant analgesics such as anticonvulsants or antidepressants, remains the main pharmacotherapeutic approach for cancer pain relief.

However, pharmacological management of cancer pain also has important limitations. NSAIDs are associated with gastrointestinal toxicity, whereas opioids may cause sedation, dependence, and respiratory depression, which can significantly affect the QoL of patients with advanced cancer. Pharmacological treatment nevertheless remains the foundation of cancer pain management and may include non-opioid analgesics (such as NSAIDs or paracetamol), opioid analgesics, and adjuvant agents (including antidepressants, anticonvulsants, topical agents, and corticosteroids). However, symptom control may remain inadequate in some patients, and treatment is often limited by adverse effects [17]. Since the pain of cancer is multidimensional, covering physical, psychosocial and spiritual domains, it should also be addressed in an integrated way beyond pharmacological paradigms. Drug based approaches have to date received the most attention yet there is growing evidence suggesting that multi-domain non-pharmacological interventions directed at body, cognitive, psychosocial and spiritual domains are important adjuncts [21-23]. Such approaches include radiotherapy, transcutaneous electrical nerve stimulation (TENS), mind-body therapies, behavioural and exercise-based interventions, physical modalities, surgical procedures, and acupuncture [10]. Chinese herbal formulas are commonly used in cancer pain management and have provided clinical benefits based on traditional Chinese internal treatments, but many patients do not tolerate the oral administration of highly concentrated herbal decoctions very well due to their high dosage or bitter taste and may also harbor concerns about exacerbating gastrointestinal toxicity. Conversely, studies are increasingly supporting acupuncture as a safe and effective therapeutic modality in the management of cancer pain, with immediate effects, low toxicities, and high acceptability among patients [24]. Acupuncture, as a unique part of Chinese medicine, is considered to have therapeutic effect through adjusting meridians and regulating Qi and blood. Acupuncture has been used for pain for centuries and is safe, non-pharmacological and has practically no side effects. It has since gained a substantial evidence base supporting its role in the management of pain associated with cancer.

The present study addresses the limited research and lack of standardized protocols for acupuncture in managing breast cancer–related pain. Applying a Knowledge Discovery in Databases (KDD) method, literature-derived acupuncture prescription data were systematically analyzed to explore acupoint selection patterns and generate a data-informed candidate acupuncture prescription. This candidate prescription was then preliminarily evaluated in a clinical setting for its analgesic effect and broader therapeutic outcomes. The integration of the KDD-derived acupuncture prescription with the WHO three-step analgesic ladder may provide a clinically relevant adjuvant approach for breast cancer-related pain management.

2 METHODS

2.1 Literature review

2.1.1 Literature sources and search strategy

From database inception to May 2024, a systematic search was performed across CNKI, VIP, WanFang, PubMed and Web of Science for clinical studies evaluating acupuncture for breast cancer-related pain. A combination of subject terms and free-text keywords was used for searches. The search strategy for Chinese databases was: ("Acupuncture" OR "Needling" OR "Electroacupuncture" OR "Needle") AND ("Cancer pain" OR "Malignant pain" OR "Breast cancer-related pain"). The English search strategy was: ("cancer pain" [Title/Abstract] OR "breast cancer-related pain" [Title/Abstract]) AND ("acupuncture" [Title/Abstract] OR "needle" [Title/Abstract] OR "electroacupuncture" [Title/Abstract] OR "acupuncture therapy" [Title/Abstract]).

2.1.2 Inclusion criteria for the literature

Studies were included if they met the following criteria:

(1) Clinical research examining acupuncture for breast cancer-related pain or cancer pain without a specific type of cancer (Studies focusing on cancer-induced pain but without specifying the type were retained due to the practical applicability of identifying frequently used acupuncture protocols for managing cancer pain that could potentially provide a reference for treating breast cancer-related pain).

(2) Participants with cancer-related pain, including breast cancer patients specifically reported.

(3) Acupuncture as the primary intervention or combined with others (e.g., pharmacological treatment).

(4) Any pain-related outcome measure including scores of the intensity of pain.

(5) Relevant dissertations or peer-reviewed articles where only one dataset with complete and usable information was found.

2.1.3 Exclusion criteria for the literature

Studies were excluded if they fulfilled any of the following criteria:

(1) Review articles, case reports, animal studies, expert opinions, or proceedings.

(2) Studies whose interventions were primarily based on scalp acupuncture, wrist–ankle acupuncture, or three-edged needle techniques.

(3) Inappropriate or incomparable intervention designs across treatment and control groups.

(4) Case reports or studies that did not include a well-defined acupuncture prescription.

(5) Articles for which the full text was not accessible.

2.1.4 Data processing

A dedicated database was constructed in Excel 2021 to compile acupuncture prescriptions for breast cancer-related pain. Acupoint extraction followed these principles: If a study reported common acupoints for multiple types of cancer pain and also listed adjunct points specific to breast cancer-related pain, both primary and adjunct points were included; If a study provided a prescription for cancer pain without specifying the cancer type, only the primary acupoints were extracted and used as potentially informative references rather than breast cancer-specific evidence.

Acupoint names were standardised according to Nomenclature and Location of Acupoints (GB/T 12346–2006); for example, "Renzhong" was standardised to "Shuigou (GV26)".

Excel 2021 was used to conduct descriptive analyses of the frequency analysis of acupoints, meridian distribution, anatomical distribution, and special acupoint types. The Apriori algorithm in SPSS Modeler 18.0 was used to carry out association rule analysis of acupoint combinations. Experimental data were analyzed and clustered using SPSS 27.0 to perform high-frequency acupoint cluster analysis and generate dendrograms.

Caution should be exercised in interpreting these acupoint patterns, as the KDD literature mining process could be affected by incomplete coverage of databases, variations in reporting, and possible publication bias.

2.2 Clinical data collection

2.2.1 Clinical case sources

Patients with breast cancer-related pain who met the inclusion criteria and were treated in the inpatient or outpatient oncology departments of Gansu University of Chinese Medicine between June 2024 and February 2025 were enrolled in this study.

2.2.2 Diagnostic criteria

(1) Western medicine diagnostic criteria: The diagnosis of breast cancer was based on medical history, physical examination, imaging studies, and pathological assessment, with definitive confirmation relying on histopathology. Diagnostic criteria followed the *Guidelines for the Diagnosis and Treatment of Breast Cancer (2022 edition)*. Pain assessment was conducted in accordance with the *Guidelines for the Diagnosis and Management of Cancer Pain (2018 edition).*

(2) Traditional Chinese Medicine (TCM) diagnostic criteria: TCM diagnosis was based on the criteria for Ruyan ("breast rock") as outlined in the *Traditional Chinese Surgery* textbook of the National TCM Higher Education "13th Five-Year Plan" series.

2.2.3 Inclusion criteria for patients

Patients were eligible for inclusion if they met the following conditions:

(1) Histopathologically confirmed diagnosis of breast cancer.

(2) Aged 30–70 years with an expected survival of more than 3 months.

(3) Karnofsky Performance Status (KPS) score >30.

(4) Receiving and consistently adhering to WHO three-step analgesic ladder during the study period.

(5) Intact verbal communication ability and capacity to complete symptom and quality-of-life assessments.

(6) Willingness to participate and provision of written informed consent.

2.2.4 Exclusion criteria for patients

Patients were excluded if they met any of the following conditions:

(1) Hypoxic respiratory depression or severe hepatic or renal insufficiency.

(2) History of syncope with needling or fear of acupuncture.

(3) Pregnancy or breastfeeding.

(4) History of breast surgery within the past month.

(5) Severe infection, ulcerated skin lesions, or communicable diseases.

(6) Long-term use of psychotropic medications.

2.2.5 Criteria for withdrawal and dropout

Patients were withdrawn or considered dropouts under any of the following conditions:

(1) Occurrence of adverse reactions during the study leading to refusal to continue treatment.

(2) Poor adherence or inability to complete the prescribed treatment course.

(3) Clinical deterioration during the intervention that rendered continuation inappropriate.

(4) Inability to continue treatment due to external or unavoidable circumstances (e.g., social or environmental factors).

2.3 Research methods

2.3.1 Sample size estimation

Sample size was calculated using the formula for comparing two independent means with equal group sizes. The Numerical Rating Scale (NRS) was selected as the primary outcome. According to published data, the mean NRS reduction after 3 weeks was 3.30 in the control group and 4.11 in the intervention group, with a standard deviation σ=1.05. With a two-sided α=0.05, β=0.20, and a group allocation ratio K=1, the required sample size was determined using the standard formula. To account for potential attrition and withdrawals, the calculated sample size was increased by 10%, resulting in a final planned enrolment of 60 participants.

The sample size formula was as follows:

$$N_1 = N_2 = \frac{(Z_\alpha + Z_\beta)^2 \sigma^2 (1 + \frac{1}{k})}{(\mu_1 - \mu_2)^2}$$

2.3.2 Randomization and control

(1) Randomization: Sixty eligible participants were randomly assigned to either the intervention or control group. Sixty

opaque, sequentially numbered envelopes were prepared corresponding to the order in which participants were enrolled. Each envelope contained a pre-generated group assignment card, and envelopes were randomly categorized into the treatment or control arm. Participants were allocated according to the group designation within their corresponding envelope. The randomisation sequence was generated using a computer-based random number table by an independent researcher.

(2) Control: Participants were divided into two study groups. Patients in the intervention group received acupuncture combined with the WHO three-step analgesic ladder, whereas those in the control group were managed with the WHO three-step analgesic regimen alone. After treatment, within-group and between-group comparisons were carried out to evaluate the efficacy and safety of acupuncture against breast cancer-related pain.

2.3.3 Treatment protocols

(1) Basic treatment: Both the intervention and control groups received standard analgesic therapy based on the WHO three-step analgesic ladder. Analgesics were selected according to the patient's baseline pain intensity: Patients with mild pain (NRS≤3) received oral celecoxib 200 mg twice daily (Approval No. H20193414, Jiangsu Chiatai Qingjiang Pharmaceutical Co., Ltd.); those with moderate pain (4≤NRS≤6) were given oral tramadol hydrochloride sustained-release tablets 100 mg twice daily (Approval No. H19980214, Mundipharma (China) Pharmaceutical Co., Ltd.); and patients with severe pain (NRS≥7) took oral oxycodone hydrochloride sustained-release tablets 20 mg twice daily (Approval No. H20140314, Mundipharma (China) Pharmaceutical Co., Ltd.). Appropriate analgesics were initially selected according to the patient's pain severity, and doses were adjusted based on clinical response and tolerability.

(2) Treatment groups: Patients in the control group were treated with the WHO three-step analgesic ladder alone, whereas those in the intervention group received conventional WHO-recommended three-step analgesia combined with acupuncture; the acupoint prescription of acupuncture was formulated based on data mining of published literatures.

(3) Treatment duration and frequency: The study comprised three treatment cycles. Each cycle consisted of five acupuncture sessions per week followed by a two-day rest period, for a total of three consecutive cycles.

(4) Acupoint selection, localization, and needling procedure (intervention group): Acupuncture was performed at bilateral Hegu (LI4), Zusanli (ST36), Neiguan (PC6), Taichong (LR3), Sanyinjiao (SP6), as well as local Ashi points, with all acupoint locations defined in accordance with the national standard Nomenclature and Location of Acupoints (GB/T 12346-2006). Disposable sterile Huacheng filiform needles (manufacturer: Keyuanda Medical Supplies Co., Ltd.; Registration No. 20172201110; Hebei Provincial Medical Products Administration Medical Device Production License No. 20220039), An'erdian III skin disinfectant and disposable sterile cotton swabs were adopted for the acupuncture procedure. All patients were maintained in the supine position with their chests and limbs adequately exposed prior to acupuncture intervention. The operator's hands were disinfected with 75% medical alcohol, followed by routine disinfection of each acupoint area using povidone-iodine swabs. Disposable sterile Huacheng filiform acupuncture needles (0.25 mm×25 mm or 0.25 mm×40 mm) were used for needling. All acupoints were treated with perpendicular insertion, with individualized depths as follows: 0.5–1.0 cun for LI4, 1.0–1.5 cun for ST36, 0.5–1.0 cun for PC6, 0.5–0.8 cun for LR3, and 1.0–1.5 cun for SP6. The insertion depth of Ashi points was adjusted based on local anatomical conditions, while tumor-involved tissues were strictly avoided during the procedure. Rapid needle insertion was performed using the three-finger needling technique. Standard lifting-thrusting and twisting-rotating manipulations were delivered to elicit the Deqi sensation, and all needles were retained for 30 minutes after manipulation completion.

2.3.4 Quality control

Case collection was conducted in strict accordance with the predefined inclusion and exclusion criteria. Acupuncture procedures were performed by operators who received standardized training and supervised instruction to ensure consistency and technical proficiency. Outcome assessments were conducted by two trained researchers to minimize measurement variability.

2.3.5 Ethical approval

This study was reviewed and approved by the Ethics Committee of the Affiliated Hospital of Gansu University of Chinese Medicine (Approval No. [2024]127).

2.3.6 Outcome measures

(1) Numerical Rating Scale (NRS): Pain intensity was recorded at baseline and after three treatment cycles using the NRS. To minimize variability, all assessments were performed by the same physician.

(2) Frequency of breakthrough cancer pain episodes (BTcP) within 24 h: Evaluated according to the *Expert Consensus on Cancer Breakthrough Pain (2019 edition)*. The same physician

Table 1. Criteria for efficacy assessment

Efficacy category	Primary symptoms and signs	Efficacy index
Complete remission	Pain disappears or is almost completely relieved	91%-100%
Partial remission	Marked reduction in pain	61%-90%
Mild remission	Slight reduction in pain, with sleep quality affected	31%-60%
No remission	No evident improvement	<31%

Note: The efficacy index is calculated as the percentage reduction in NRS score from baseline to post-treatment. NRS, Numerical Rating Scale.

documented the number of episodes before treatment and after three cycles to capture dynamic changes in pain.

(3) Duration of pain relief: Used to assess the effectiveness of the treatment regimen. The duration of pain relief following analgesic treatment, defined as the patient-reported time interval during which noticeable pain reduction was maintained, was recorded by the same physician at baseline and after three treatment cycles.

(4) Karnofsky Performance Status (KPS): The KPS scale was used to evaluate functional status, with higher scores indicating better overall health. Because of the subjective nature of this measure, the same physician assessed KPS before and after the intervention.

(5) Pittsburgh Sleep Quality Index (PSQI): A self-administered questionnaire assessing sleep quality over the preceding month. Scores were collected by the same physician at baseline and following three treatment cycles. Given that the PSQI assesses sleep quality over the previous month, the post-treatment PSQI collected after the 3-week intervention mainly reflected sleep during the intervention period but also partially overlapped with the pre-intervention baseline window.

(6) Self-Rating Depression Scale (SDS): Based on the *Chinese Guideline for Prevention and Treatment of Depressive Disorders (2nd edition)*, the SDS is a 20-item self-report tool assessing emotional and somatic symptoms of depression. Higher scores indicate greater depressive severity. The same physician recorded scores before and after three treatment cycles.

2.3.7 Criteria for efficacy assessment

Therapeutic efficacy was evaluated according to the degree of pain relief, with the efficacy index defined as the percentage reduction in NRS score from baseline to post-treatment (see **Table 1**).

2.3.8 Adverse event monitoring

Adverse events related to acupuncture-such as needling discomfort, vasovagal reactions, bleeding, subcutaneous hematoma, local erythema, or blistering at acupoint sites-were recorded. Patients were also monitored for potential adverse reactions to WHO three-step analgesics ladder, including constipation, drowsiness, dizziness, dry mouth, and nausea or vomiting. All events were documented, and appropriate management measures were implemented as needed.

2.3.9 Statistical analysis

Statistical analyses were performed using SPSS version 27.0. For continuous variables that met assumptions of normality and homogeneity of variance, independent-sample t tests were used for between-group comparisons, and paired *t* tests for within-group comparisons. Normality was assessed separately for each variable at each time point using appropriate tests, and the choice of parametric or non-parametric tests was made accordingly. When variances were unequal, adjusted *t* tests were applied, and data were expressed as mean ± standard deviation $\bar{x}\pm s$. For non-normally distributed data, outcomes were reported as median and interquartile range [M (P25, P75)]. Categorical variables were analysed using the chi-square test or Fisher's exact test, as appropriate, and ordinal data were assessed using rank-sum tests. Differences were considered statistically significant at $P<0.05$ and highly significant at $P<0.01$. The primary analysis was conducted on participants who completed the study (per-protocol population). Given the exploratory nature of this study and the relatively small sample size, no formal adjustment for multiple comparisons was performed.

3 RESULTS

3.1 Literature screening

A total of 161 studies met the predefined inclusion and exclusion criteria, yielding 161 acupuncture prescriptions encompassing 92 distinct acupoints and 614 cumulative usages, excluding Ashi points. Among all acupoints, including Ashi points, 28 appeared ≥5 times. Among them, the top ten acupoints were Hegu (LI4): 92 times; Zusanli (ST36): 83 times; Ashi points: 65 times; Neiguan (PC6): 63 times; Taichong (LR3): 49 times; Sanyinjiao (SP6): 47 times; Quchi (LI11): 16 times; Baihui (DU20): 12 times; Guanyuan (RN4): 10 times and Qihai (RN6): 9 times (**Table 2**). Seventy-seven special-acupoint category assignments were identified in 161 prescrip-

Table 2. Analysis of acupoint frequency

Acupoint	Frequency	Frequency percentage (%)	Acupoint	Frequency	Frequency percentage (%)
Zusanli (ST36)	83	51.55	Dazhu (BL11)	7	4.35
Ashi points	65	40.37	Shenshu (BL23)	7	4.35
Neiguan (PC6)	63	39.13	Xuehai (SP10)	7	4.35
Taichong (LR3)	49	30.43	Baxie (EX-UE9)	6	3.73
Sanyinjiao (SP6)	47	29.19	Dazhui (DU14)	6	3.73
Quchi (LI11)	16	9.94	Geshu (BL17)	6	3.73
Baihui (DU20)	12	7.45	Kongzui (LU6)	6	3.73
Guanyuan (RN4)	10	6.21	Rugen (ST18)	6	3.73
Qihai (RN6)	9	5.59	Xuanzhong (GB39)	6	3.73
Liangqiu (ST34)	8	4.97	Mingmen (DU4)	5	3.11
Waiguan (SJ5)	8	4.97	Danzhong (RN17)	5	3.11
Hegu (LI4)	92	57.14	Bafeng (EX-LE10)	7	4.35
Yanglingquan (GB34)	8	4.97	Taixi (KI3)	5	3.11
Zhongwan (RN12)	8	4.97	Yinlingquan (SP9)	5	3.11

Note: Frequency percentage is calculated as frequency/number of prescriptions ×100%. Acupoint codes follow international standard nomenclature. BL, Bladder meridian; DU, Governor Vessel; EX, Extra points; GB, Gallbladder meridian; HT, Heart meridian; KI, Kidney meridian; LI, Large Intestine meridian; LR, Liver meridian; LU, Lung meridian; PC, Pericardium meridian; RN, Conception Vessel; SI, Small Intestine meridian; SJ, Sanjiao meridian; SP, Spleen meridian; ST, Stomach meridian.

Table 3. Analysis of specific acupoints

Specific acupoint	Frequency (%)	Number of acupoints (%)	Acupoint (frequency)
Five Shu-stream acupoint	188 (23.36)	14 (18.18)	ST36 (83); LR3 (49); LI11 (16)
Yuan-source acupoint	152 (18.89)	5 (6.49)	LI4 (92); LR3 (49); Shenmen (HT7) (5)
Crossing acupoint	112 (13.91)	22 (28.57)	SP6 (47); DU20 (12); RN4 (10)
Lower He Sea acupoint	94 (11.68)	3 (3.90)	ST36 (83); GB34 (8); Weizhong (BL40) (3)
Eight confluent acupoint	80 (9.94)	7 (9.09)	PC6 (63); SJ5 (8); Houxi (SI3) (4)
Luo-connecting acupoint	79 (9.81)	5 (6.49)	PC6 (63); SJ5 (8); Fenglong (ST40) (4)
Eight influential acupoint	42 (5.22)	6 (7.79)	RN12 (8); GB34 (8); BL11 (7)
Front-mu acupoint	30 (3.73)	7 (9.09)	RN4 (10); RN12 (8); RN17 (5)
Back-shu acupoint	28 (3.48)	8 (10.39)	BL23 (7); BL17 (6); Ganshu (BL18) (4)

Note: The "Acupoint (frequency)" column lists the top three acupoints by frequency within each category. The percentage in the "Frequency (%)" column was calculated based on the total frequency of special acupoint usage (n=805), whereas the percentage in the "Number of acupoints (%)" column was calculated based on the total number of special acupoints identified (n=77).

tions, with a total usage frequency of 805 (**Table 3**). The Five-Shu Stream points, Yuan-Source points and Crossing points were the three most commonly used categories which appeared together 452 times (56.16%). Among these categories, Crossing points, represented mainly by SP6, accounted for a large proportion, while Five-Shu Stream points were mainly represented by ST36 and LR3.

Apart from Ashi points, the acupoints were allocated across 14 meridians and extra-point categories, as shown in **Table 4**. The ST, LI and PC meridians were the most commonly used (287 uses: 46.90%). The largest numbers of distinct acupoints were found in the Bladder (BL), ST, and Governor Vessel (DU) meridians, whereas the Heart (HT) and Small Intestine (SI) meridians were the least represented (**Table 4**).

Regarding anatomical distribution, acupoints located on the upper and lower limbs were most frequently used, involving 45 acupoints with a combined frequency of 471 (76.71%). This was followed by lumbar-back and abdominal regions, comprising 28 acupoints with a total of 85 uses (13.84%) (**Table 5**).

Association rule mining in SPSS Modeler 18.0 was performed using a minimum support of 10%, minimum confidence of 80%, and a maximum of two antecedent acupoints. Ten acupoint combinations were identified. The combination with the highest support was LI4-LR3, followed by ST36-SP6 and LI4-SP6 (**Table 6**). In association rule mining, the antecedent indi-

Table 4. Analysis of meridian frequency

Meridian	Frequency (%)	Number of acupoints (%)	Acupoint (frequency)
Stomach (ST)	112 (18.30%)	12 (13.04%)	ST36 (83); ST34 (8); ST18 (6)
Large Intestine (LI)	110 (17.97%)	4 (4.35%)	LI4 (92); LI11 (16); Binao (LI14) (1)
Pericardium (PC)	65 (10.62%)	5 (5.43%)	PC6 (63)
Spleen (SP)	60 (9.80%)	5 (5.43%)	SP6 (47); SP10(7); SP9 (5)
Liver (LR)	59 (9.64%)	4 (4.35%)	LR3 (49); Qimen (LR14) (4); Zhongdu (LR6) (4)
Bladder (BL)	43 (7.03%)	15 (16.30%)	BL11 (7); BL23 (7); BL17 (6)
Conception Vessel (RN)	36 (5.88%)	7 (7.61%)	RN4 (10); RN6 (9); RN12 (8)
Governor Vessel (DU)	34 (5.56%)	9 (9.78%)	DU20 (12); DU14 (6); DU4 (5)
Extra point	21 (3.43%)	7 (7.61%)	EX-LE10 (7); EX-UE9 (6); Jiaji Points (4)
Gallbladder (GB)	21 (3.43%)	8 (8.70%)	GB34 (8); GB39 (6); Jianjing (GB21) (2)
Sanjiao (SJ)	15 (2.45%)	4 (4.35%)	SJ5 (8); Zhigou (SJ6) (4); Sanyangluo (SJ8) (1); Jianliao (SJ14) (2)
Lung (LU)	12 (1.96%)	4 (4.35%)	LU6 (6); Chize (LU5) (3); Lieque (LU7) (2)
Kidney (KI)	9 (1.47%)	5 (5.43%)	KI3 (5); Fuliu (KI7) (1); Shenfeng (KI23) (1)
Heart (HT)	8 (1.31%)	3 (3.26%)	HT7 (5); Jiquan (HT1) (2); Yinxi (HT6) (1)
Small Intestine (SI)	7 (1.14%)	4 (4.35%)	SI3 (4); Jianzhen (SI9) (1); Shaoze (SI1) (1)

Note: The "Acupoint (frequency)" column lists the three most frequently used acupoints for each meridian rather than all acupoints identified in the prescriptions.

Table 5. Analysis of the locations of acupoints

Part	Frequency (%)	Number of acupoints (%)	Acupoint (frequency)
Lower extremities	248 (40.39%)	23 (25.00%)	ST36 (83); LR3 (49); SP6 (47)
Upper extremities	223 (36.32%)	22 (23.91%)	LI4 (92); PC6 (63); LI11 (16)
Back	43 (7.04%)	16 (17.39%)	BL23 (7); BL17 (6); DU4 (5)
Abdomen	42 (6.84%)	12 (13.04%)	RN4 (10); RN6 (9); RN12 (8)
Head and neck	34 (5.54%)	9 (9.78%)	DU20 (12); BL11 (7); DU14 (6)
Chest	17 (2.77%)	7 (7.61%)	ST18 (6); RN17 (5); Yingchuang (ST16) (2)
Face	7 (1.14%)	3 (3.26%)	Shuigou (DU26) (3); Yintang (EX-HN3) (3); Cuanzhu (BL2) (1)

Note: The 'acupoint (frequency)' column lists the top three acupoints by frequency for each anatomical region. The percentage in the 'Number of acupoints (%)' column was calculated based on the total number of acupoints identified (n=92).

Table 6. Analysis of acupoint association rules

Number	Consequent	Antecedent	Support (%)	Confidence (%)
1	LI4	LR3, PC6	11.18	94.74
2	LI4	LR3, Ashi points	10.56	94.44
3	LI4	LR3, ST36	14.91	92.31
4	LI4	PC6, ST36	22.36	90.00
5	LI4	LR3	27.33	89.80
6	LI4	SP6, ST36	21.73	89.74
7	ST36	SP6, LI4	21.73	89.74
8	LI4	SP6, PC6	15.53	86.21
9	ST36	SP6	24.22	82.98
10	LI4	SP6	24.22	82.99

Note: Association rules were generated using the Apriori algorithm with minimum support of 10% and minimum confidence of 80%. "Antecedent" indicates the preceding acupoint(s), and "Consequent" indicates the co-occurring acupoint.

cates preceding acupoint(s), while the consequent identifies the co-occurring acupoint. A complex-network relationship diagram is shown in **Figure 1**.

Cluster analysis was conducted on acupoints used ≥5 times using SPSS 27.0. The hierarchical dendrogram (**Figure 2**) and cluster membership plot (**Figure 3**) indicated that 28 acupoints clustered into three major groups:

Cluster 1: EX-UE9, EX-LE10, LI11, SJ5, GB34, SP9, LI4, LR3, ST36, SP6, PC6

Cluster 2: BL11, GB39, SP10, KI3, DU4, BL23, DU14, LU6, ST34, Ashi points, DU20, ST18, RN17, RN4, RN6, RN12

Cluster 3: BL17

These findings suggest that acupoint selection for breast cancer-related pain may exhibit identifiable patterns, with LI4, ST36, PC6, LR3, and SP6 emerging as frequently used candidate core points and limb acupoints showing predominance across included prescriptions.

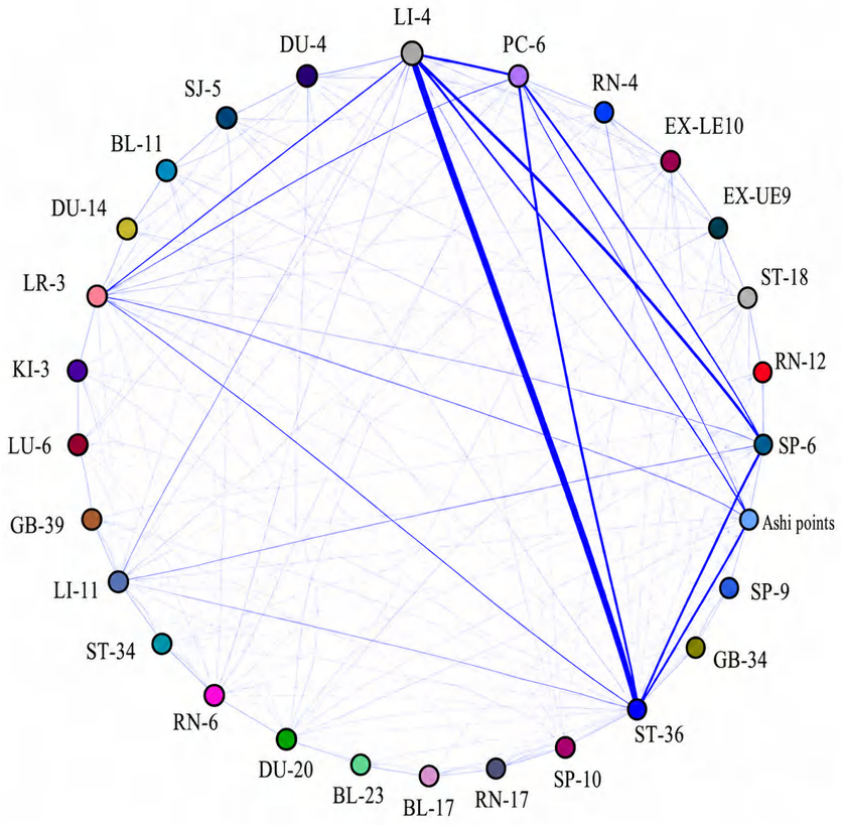

Figure 1. Complex network of acupoint co-occurrence based on association rule mining. Nodes represent acupoints and edges indicate co-occurrence relationships; Association rules were generated with a minimum support of 10% and a minimum confidence of 80%.

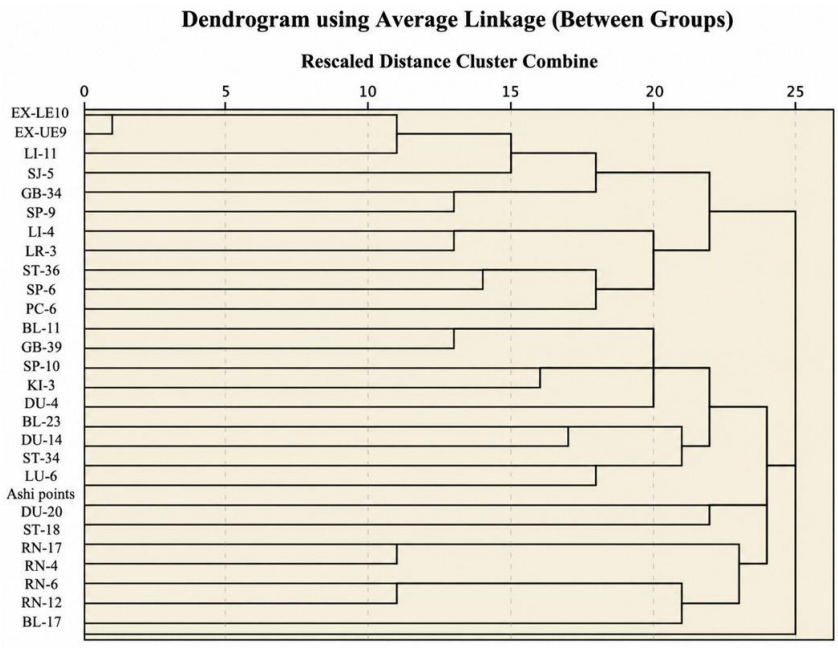

Figure 2. Hierarchical clustering dendrogram of high-frequency acupoints (≥5 occurrences). The dendrogram illustrates similarity relationships among frequently used acupoints; a lower linkage height indicates greater similarity.

3.2 Clinical study findings

A total of 60 patients meeting the inclusion and exclusion criteria were enrolled, with 30 allocated to the intervention group and 30 to the control group. During treatment, two patients in the control group withdrew due to disease progression that prevented completion of follow-up assessments, resulting in 30 evaluable cases in the intervention group and 28 in the control group for per-protocol analysis. Baseline characteristics, including age, disease duration and NRS scores, were normally distributed and showed homogeneity of variance. Independent-sample t tests demonstrated no significant between-group differences (P>0.05), indicating good baseline comparability. Pain locations-distributed across the chest, abdomen, head, lumbar region, shoulder–back region and limbs-also showed no significant group differences (χ^2 test, P>0.05; **Table 7**). Initial analgesic use, based on the WHO three-step analgesic ladder, was similarly comparable between groups (χ^2 test, P>0.05; **Table 8**).

Both groups exhibited significant reductions in NRS scores following treatment, with the intervention group showing a greater mean reduction (3.37±1.27) than the control group (2.18±0.90). Although baseline NRS scores did not differ significantly between groups, post-treatment values were significantly lower in the intervention group (Mann-Whitney U test, P<0.05), and the between-group difference in NRS change scores was also statistically significant (P<0.05; **Table 9**), suggesting better short-term analgesic outcomes with adjunctive acupuncture.

Likewise, the frequency of 24-hour breakthrough pain episodes decreased significantly within both groups (intervention: Z=-4.434, P<0.001; control: Z=-4.350, P<0.001). Baseline values were similar but post-treatment measures were significantly lower in the intervention group (P<0.05; **Table 10**). The duration of pain relief was significantly prolonged in both groups post-treatment, with the intervention group demonstrating a significantly longer duration compared to the control group (Mann-Whitney U test, P<0.05; **Table 11**).

Sleep quality, assessed by PSQI, improved significantly in both groups. The control group showed significant improvement using paired t testing (P<0.001), whereas the intervention

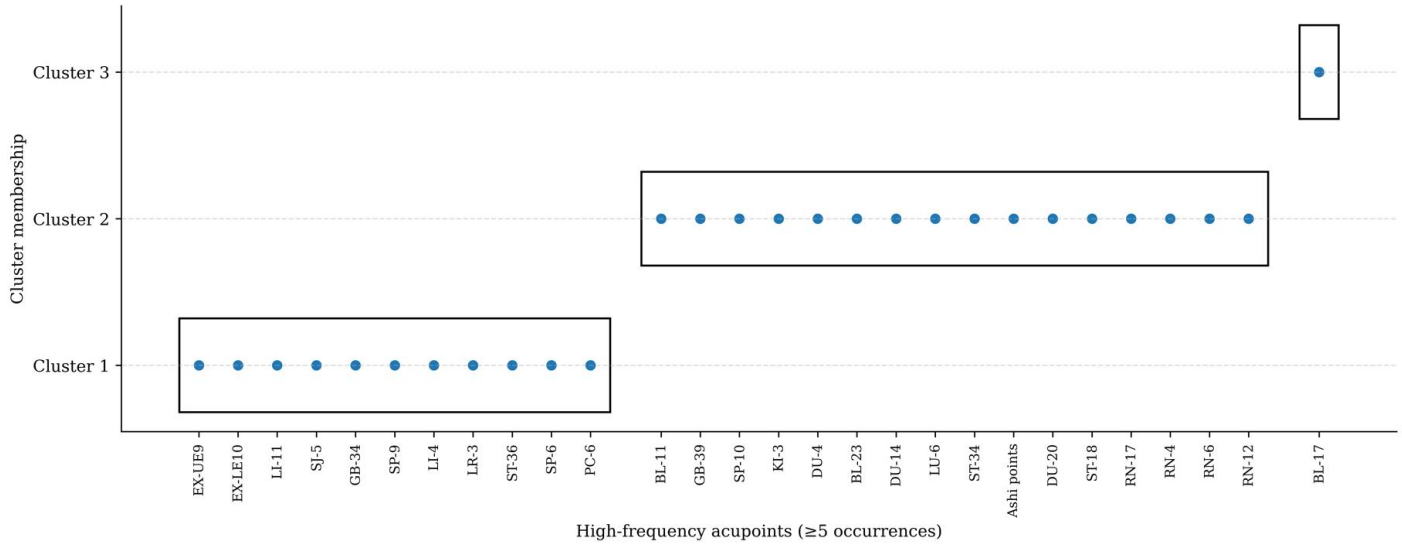

Figure 3. Cluster membership plot of hierarchical clustering results for high-frequency acupoints (≥5 occurrences). The plot summarizes the three-cluster membership pattern identified by hierarchical clustering and corresponds to the cluster groups described in the main text.

Table 7. Comparison of pain locations between the two groups (n)

Group	Abdomen	Shoulder-back	Head	Chest	Waist	Limbs
Treatment group (n=30)	5	5	4	6	5	5
Control group (n=28)	4	4	4	4	5	7

Note: Data are presented as number of cases (n). Between-group comparisons showed no significant difference (χ^2=1.055, P=0.983). Statistical analyses were performed using the chi-square test or Fisher's exact test, as appropriate. A two-sided *P*<0.05 was considered statistically significant. χ^2, chi-square test.

Table 8. Comparison of initial analgesic use between the two groups [n (%)]

Group	Celecoxib	Tramadol	Oxycodone	χ^2	*P*
Treatment group (n=30)	6 (20.0)	17 (56.7)	7 (23.3)	0.056	0.972
Control group (n=28)	6 (21.4)	15 (53.5)	7 (25.0)		

Note: Data are presented as n (%). Between-group comparisons were performed using the chi-square test (χ^2). A two-sided *P*<0.05 was considered statistically significant.

Table 9. Comparison of NRS scores before and after treatment in the two groups

Group	Pretreatment NRS	Posttreatment NRS	*T/Z*	*P*	Difference (Δ)
Treatment group	5.20±1.69	2 (1, 2)	-4.817#	<0.001	3.37±1.27
Control group	5.05±1.75	3 (1.25, 4)	-4.821#	<0.001	2.18±0.90
T/Z	0.363*	-2.475#			-3.645#
P	0.718	0.013			<0.001

Note: Data are presented as mean ± SD or median (P25, P75), as appropriate. Δ indicates the change from baseline to post-treatment and is presented as mean ± SD for descriptive purposes. # indicates Wilcoxon signed-rank test or Mann–Whitney U test, as appropriate; * indicates Student's *t* test. A two-sided *P*<0.05 was considered statistically significant. SD, standard deviation; Δ, change from baseline.

group showed significant improvement using rank-sum testing (*P*<0.001). Baseline PSQI scores were comparable (*P*>0.05), but post-treatment scores were significantly lower in the intervention group (Mann–Whitney U test, *Z*=-3.520, *P*<0.001; **Table 12**). Both groups also showed significant reductions in SDS scores (intervention: *Z*=-4.791; control: *Z*=-4.641; both *P*<0.05), with post-treatment SDS values significantly lower in the intervention group (rank-sum test, *P*<0.05; **Table 13**).

KPS scores increased significantly in both groups (intervention: *P*<0.01; control: *P*<0.05). While baseline values did not differ significantly, post-treatment KPS scores were significantly higher in the intervention group (rank-sum test, *P*<0.05; **Table 14**), indicating greater improvement in functional status and quality of life. After three treatment cycles, the total effective rate was 86.67% in the intervention group and 71.43% in the control group. Comparison of the ordinal efficacy categories showed a significant difference between groups (*Z*=-3.392, *P*=0.001; **Table 15**), suggesting that acupuncture combined with standard analgesics was associated with better overall therapeutic outcomes than analgesics alone.

In summary, acupuncture with pharmacotherapy had stable advantages in the clinical endpoints rather than the improvement of a single outcome. Aside from a higher intensity of pain reduction, subjects who received adjunctive acupuncture also reported fewer breakthrough pain episodes and more prolonged analgesia as well as short-term improvements in functional status, sleep quality during the treatment phase, and emotional well-being. These findings

Table 10. Comparison of 24-hour breakthrough pain episodes before and after treatment between the two groups [M (P25, P75)]

Group	Pretreatment	Posttreatment	*Z*	*P*
Treatment group	4 (2, 5)	1 (0, 2)	-4.434	<0.001
Control group	4 (3, 5)	2 (1, 3)	-4.350	<0.001
T/Z	-0.556	-2.078		
P	0.578	0.038		

Note: Data are presented as median (P25, P75). Within-group comparisons were performed using Wilcoxon signed-rank test; between-group comparisons were performed using Mann–Whitney U test, as appropriate. A two-sided $P<0.05$ was considered statistically significant. BTcP, breakthrough cancer pain.

Table 11. Comparison of pain-relief duration before and after treatment between the two groups

Group	Pretreatment	Posttreatment	*Z*	*P*
Treatment group	4.13±1.48	7 (6, 8)	-4.993#	<0.001
Control group	4.25±1.43	5 (4.25, 6)	-4.564#	<0.001
T/Z	-0.305*	-2.685#		
P	0.762	0.007		

Note: Data are presented as mean ± SD or median (P25, P75), as appropriate. # indicates Wilcoxon signed-rank test or Mann–Whitney U test, as appropriate; * indicates Student's *t* test. A two-sided $P<0.05$ was considered statistically significant.

Table 12. Comparison of PSQI scores before and after treatment between the two groups

Group	Pretreatment	Posttreatment	*T/Z*	*P*
Treatment group	13.40±1.94	9 (7, 10)	-4.863#	<0.001
Control group	13.18±2.16	10 (9, 12)	-4.912#	<0.001
T/Z	0.411*	-3.520#		
P	0.683	<0.001		

Note: Data are presented as mean ± SD or median (P25, P75), as appropriate. # indicates Wilcoxon signed-rank test or Mann–Whitney U test, as appropriate; * indicates Student's *t* test. A two-sided $P<0.05$ was considered statistically significant. PSQI, Pittsburgh Sleep Quality Index.

Table 13. Comparison of SDS scores before and after treatment between the two groups

Group	Pretreatment	Posttreatment	*Z*	*P*
Treatment group	59.03±5.65	45 (41, 50)	-4.791#	<0.001
Control group	58.46±6.77	49.5 (43, 57.5)	-4.641#	<0.001
T/Z	0.348*	-2.024#		
P	0.729	0.043		

Note: Data are presented as mean ± SD or median (P25, P75), as appropriate. # indicates rank-sum test; * indicates Student's t test. A two-sided $P<0.05$ was considered statistically significant. SDS, Self-Rating Depression Scale.

3.3 Safety evaluation

Safety analysis showed that all adverse events occurred as single incidents, with no patient experiencing more than one type of reaction. In the intervention group, constipation was reported in three cases and dizziness in two cases, with no instances of nausea or vomiting, resulting in an overall adverse event rate of 16.67%. In contrast, the control group reported eight cases of constipation, three cases of dizziness, and three cases of nausea or vomiting, yielding an overall incidence of 50.00%. Statistical comparison showed a lower incidence of adverse events in the intervention group than in the control group ($P<0.05$; **Table 16**). However, because analgesic doses were adjusted according to clinical response and cumulative analgesic exposure was not quantitatively analyzed, the safety findings should be interpreted cautiously.

4 DISCUSSION

Breast cancer continues to be a major threat to women's health worldwide, and its burden is rising, especially in low- and middle-income countries [25]. Cancer pain, one of the most common complications in advanced disease, is primarily treated through the use of the WHO three-step analgesic ladder. However, not all patients embrace long-term opioid use because of constipation, respiratory depression, and dependence. In TCM, the pathogenesis of tumors is often related to qi stagnation, phlegm accumulation and blood stasis obstructing the meridians, leading to clinical symptoms such as cancer pain [26]. As an important external therapy in TCM, acupuncture has multidimensional and multitarget regulation functions and is widely used for analgesia because of its simplicity, safety and low cost.

According to previous fundamental studies, electroacupuncture or wrist-ankle acupuncture has been reported to modulate the descending pain regulatory system, including increased release of endogenous opioid peptides such as β-endorphin and enkephalin, regulation of serotonergic activity, and activation of μ-opioid receptor-mediated pathways [27, 28]. These mechanisms are considered to contribute to the analgesic effects observed in animal models of bone cancer pain [27]. Modern neurobiological studies have indicated that acupuncture analgesia may involve multiple central and peripheral mechanisms, including the modulation of pain perception and endogenous opioid-mediated analgesic

were associated with a higher overall response rate and a lower incidence of adverse events, suggesting a multidimensional supportive role of acupuncture. However, because cumulative analgesic exposure was not quantitatively analyzed, safety outcomes should be interpreted with caution. Overall, the available evidence suggests that acupuncture may be a potentially useful adjunct in the management of cancer-related symptoms.

Table 14. Comparison of KPS scores before and after treatment between the two groups [M (P25, P75)]

Group	Pretreatment	Posttreatment	*Z*	*P*
Treatment group	70 (60, 70)	80 (70, 80)	-4.767	<0.001
Control group	70 (60, 70)	70 (70, 80)	-3.742	<0.001
Z	-0.800	-3.154		
P	0.936	0.002		

Note: Data are presented as median (P25, P75). Between-group comparisons were performed using rank-sum test. A two-sided $P<0.05$ was considered statistically significant. For KPS, quartile values were presented using the nearest valid scale category because the scale is recorded in 10-point increments. KPS, Karnofsky Performance Status.

pathways [29]. Based on these mechanistic insights, this study evaluated the clinical outcomes and safety of acupuncture combined with three-step analgesic therapy in patients with breast cancer-related pain using NRS scores, frequency of breakthrough pain, duration of pain relief, KPS, PSQI, and SDS assessments.

Through KDD analysis, we identified an acupoint profile potentially relevant to the management of breast cancer-related pain. High-frequency acupoints included LI4, ST36, Ashi points, PC6, and LR3 distributed primarily on the upper and lower limbs which reflects a combination of distal points with local Ashi points. The dominant involved meridians are ST and Large Intestine meridian, both characterized by abundant qi and blood, together with Pericardium meridian closely related to the maintenance of emotional regulation. The most commonly used among special acupoints are the Five-Shu, Yuan-Source and crossing points. Association rule mining identified recurrent acupoint associations, including combinations centered on LI4, LR3, PC6, ST36, and SP6. Cluster analysis grouped the high-frequency acupoints into several clinically interpretable categories. Together, these findings supported the formulation of a candidate acupuncture prescription aimed at harmonizing qi and blood and relieving pain.

Previous clinical studies of acupuncture for cancer-related pain have generally demonstrated that acupuncture interventions can decrease the intensity of the pain experience, against a background of both anecdotal reports and trial results suggesting additional quality-of-life–related benefits including sleep impact and emotional status, and overall the findings from this study align with this evidence trend [30, 31]. Nonetheless, the heterogeneity of outcomes across studies has often been high due to variation in intervention variables and yet clinical protocols are not tightly regulated for consistency and comparability [32]. Against this background, the novelty of the current study lies in proposing a method to derive a relatively structured candidate acupoint prescription systematically from literature data within a KDD framework before preliminary clinical evaluation, thereby establishing a "data-mining–to–clinical-evaluation" research pathway. Methodologically, this approach may help reduce heterogeneity in acupoint selection and improve the reproducibility and clinical applicability of acupuncture interventions. Integratively, combining this evidence-derived acupuncture prescription system with the WHO three-step analgesic ladder may yield an interesting structured and spatiotemporal guiding basis for application as well as a systematic approach complementary to the management of breast cancer–related pain.

Anatomically and physiologically, the breast is associated with multiple meridians, especially ST and Liver meridians; therefore, treating any related zang-fu organs can affect breast diseases. LI4, via Yuan-Source point of Large Intestine meridian, regulates qi and frees channels. Its pairing with LR3, known as the "Four Gates", when combined, harmonizes qi and blood and directly addresses the TCM principle of "no obstruction, no pain". In this regard, ST36 (He-Sea point of ST meridian), combined with SP6, tonifies qi and blood, strengthens the Spleen/Liver/Kidney systems, thus acting on the principle that "when nourished there is no pain". Ashi points, in accordance with the dictum "where there is pain, there is a point", work directly where the problem lies by dispersing stagnation and quickly relieving symptoms. PC6 belongs to the Pericardium meridian and connects with the Sanjiao, relieving chest oppression while also regulating qi and mitigating emotional distress. Given that the pericardial pathway is traditionally considered related to the breast region, PC6 may offer potential benefits for breast cancer-related pain and related psychological symptoms.

The present study sought to develop and evaluate a KDD-based candidate acupoint prescription in a randomized controlled trial. The combined treatment was associated with greater reductions in pain scores, fewer episodes of BTcP, and a longer duration of pain relief compared with standard analgesic therapy alone. Improvements were also observed in functional status, sleep quality, and depressive symptoms. In addition, a lower incidence of adverse events was observed in the combined-treatment group, and no acupuncture-related adverse events were recorded. However, because analgesic doses were adjusted according to clinical response and cumulative analgesic exposure was not quantitatively analyzed, this safety finding should be interpreted cautiously. Overall, these findings suggest that acupuncture may serve as a potentially useful adjunctive therapy for alleviating breast cancer-related pain.

Although the present study yielded encouraging clinical outcomes, several limitations should be acknowledged. These constraints are generally consistent with findings from previous systematic reviews, which have noted that clinical studies of acupuncture for cancer-related pain are often characterized by relatively small sample sizes and considerable methodological heterogeneity [33]. This study had a small clinical sample size, with 60 patients enrolled and 58 completing the trial (30

Table 15. Comparison of overall treatment efficacy between the two groups after therapy [n (%)]

Group	Number of cases	Complete remission	Partial remission	Mild remission	No remission	Total effective rate	*Z*	*P*
Treatment group	30	4 (13.33)	20 (66.67)	2 (6.67)	4 (13.33)	86.67%	-3.392	0.001
Control group	28	1 (3.57)	7 (25.0)	12 (42.86)	8 (28.57)	71.43%		

Note: Data are presented as n (%). Between-group comparisons were performed using rank-sum test; *Z* denotes the test statistic. A two-sided $P<0.05$ was considered statistically significant. The four-level efficacy outcomes were treated as ordinal data and analyzed using the rank-sum test. *Z*, test statistic.

Table 16. Safety evaluation during treatment in the two groups

Group	Number of cases	Constipation	Dizziness	Nausea and vomiting	No adverse reactions	Overall incidence	*P*
Treatment group	30	3	2	0	25	16.67%	0.036
Control group	28	8	3	3	14	50.00%	

Note: Data are presented as number of cases (n) unless otherwise specified. Between-group comparisons were performed using Fisher's exact test. A two-sided $P<0.05$ was considered statistically significant. Overall incidence was defined as the proportion of patients experiencing at least one adverse event.

and 28, respectively). In addition, two participants in the control group withdrew due to disease progression, and the analysis was conducted on the per-protocol population, which may have introduced potential bias. One limitation is that the literature-mining dataset contained some studies related to cancer pain without specifying the cancer type. These prescriptions were retained only as a potentially informative reference, and the acupoint patterns identified cannot be construed to be evidence for specific therapy for breast cancer. In addition, since the PSQI identifies sleep quality in the last month, the post-treatment result collected following the three-week procedure partially overlapped with the baseline assessment period, potentially impacting the precision of all interpretation associated with sleep. Thus, this relatively small sample size may have compromised the statistical power and thus, to a certain extent, the external validity of some of the findings. Future studies should be multicenter randomized controlled trials with larger sample sizes and more rigorous methodological designs to improve the stability and generalizability of these findings.

The primary outcome measures (NRS, PSQI, and SDS) were mostly determined using subjective self-reported assessments. Though these instruments are widely validated, by nature the subjective aspect may introduce measurement bias. Moreover, the lack of concurrent objective biological measurements—such as inflammatory cytokine profile monitoring, neuroimmune biomarkers, or pain-related molecular markers—complicates mechanistic interpretation at a deeper level. The inclusion of objective treatment evaluation tools and biomarker analyses, alongside patient-reported outcomes, would contribute to the scientific rigor and robustness of acupuncture analgesia research [34].

5 CONCLUSION

The results obtained in this trial show a beneficial adjuvant effect of acupuncture (performed according to the KDD-derived candidate prescription—LI4, ST36, Ashi points, PC6, LR3 and SP6) when given in conjunction with standard analgesic treatment based on the WHO three-step analgesic ladder for patients suffering from breast cancer-related pain. However, there are some limitations that should be noted. It is plausible that the KDD process was impacted by limitations in the retrieval of literature and the coverage of databases specifically relevant to acupuncture and cancer pain research. Moreover, the clinical trial had a small number of subjects and a short intervention duration of only 3 weeks with no long-term follow-up to assess the durability and sustainability of therapeutic effects. Outcome measures were mostly subjective rating scales, and biological markers were absent. The exact mechanisms involved in acupuncture analgesia are still only partially understood.

Larger multicenter studies with longer follow-up are warranted to assess the long-term therapeutic effects of acupuncture. Mechanistic investigations, such as animal models, behavioral assessments, molecular profiling and multi-omics approaches, may aid in delineating the neuroimmune regulatory pathways and important molecular targets contributing to acupuncture-elicited analgesia. This work, in turn, may ultimately lay the groundwork to enable standardized and evidence-informed acupuncture interventions for women with breast cancer to be developed and rationally integrated into routine clinical pain management.

DECLARATIONS

Author contributions

Zhaoyu Li, Yali Wei, and Jinxia Bai contributed equally to this work. Zhaoyu Li, Yali Wei, and Jinxia Bai conducted the study, collected and analyzed the data, and drafted the manuscript. Lingna Ma and Kai Sun contributed to data interpretation and manuscript revision. Wei Wang conceived and supervised the study and critically revised the manuscript. All authors have read and approved the final version of the manuscript.

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Data availability

The data supporting the findings of this study are available within the article.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Affiliated Hospital of Gansu University of Chinese Medicine (Approval No. [2024]127). Written informed consent was obtained from all participants.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflicts of interest.

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Perioperative Precision Medicine

Zentime

REVIEW ARTICLE

Unveiling the complex network of sepsis and cytokine storms: New perspectives from mechanisms to interventions

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Abstract

Sepsis is a systemic inflammatory response syndrome triggered by infection. It presents with simultaneous overactive immune activation and immune paralysis, leading to a cytokine storm, tissue injury, and multiple organ failure. The molecular mechanisms and pathological characteristics of the septic cytokine storm are comprehensively interpreted from multiple perspectives, including immune regulation, signaling pathways, cellular interactions, metabolic state reprogramming, and microcirculatory dysfunction. The cross-activation of signaling pathways, including NF-κB, JAK/STAT, mitogen-activated protein kinase (MAPK), and NLRP3 inflammasome, underlies the core mechanism for the enhancement of the inflammatory response via continued release of pro-inflammatory cytokines and also is involved in maintaining immune balance. In the late-stage immunosuppressive phase, Treg cells, myeloid-derived suppressor cells (MDSCs) and regulatory macrophages play pivotal roles in reestablishing host homeostasis through negative feedback, and their dysfunction is closely associated with secondary infections and high mortality. This review consolidates the latest developments in precision medicine and multidimensional biomarkers, emphasizing the prospective value of several markers such as lactate and CD64, pentraxin 3 (PTX3), and BMP9 for early diagnosis and prognosis evaluation. Moreover, it reviews novel immunomodulators, for instance, bee venom peptides, GDF15, N-(1,3,4-oxadiazol-2-yl) guanidine (NWG), 4-octyl itaconate (4-OI), and nanoparticle drug delivery systems that inhibit inflammatory signaling cascades, restore energy metabolism, and provide organ protection. Taken together, the pathogenic mechanisms of sepsis are non-linear and dynamic, which may render single-target therapy insufficient for successful management. Personalized interventions targeting multi-target network regulation based on omics and AI models may accomplish precision monitoring and stratified treatment of inflammatory responses, also providing new theoretical and clinical strategies to reduce sepsis-related mortality.

Keywords: Sepsis, Cytokine storm, Immune regulation, Metabolic reprogramming, NLRP3 inflammasome, Precision medicine, Biomarkers, Immune suppression

1 INTRODUCTION

Sepsis is an infection-induced systemic inflammatory response syndrome. Overactivation of the immune system during its progression results not only in hyper-inflammatory responses but may also be associated with immunosuppression, known as immune paralysis, which makes treatment difficult. However, therapies to decrease immune activation may have deleterious

effects on patients who are immunoparalyzed due to viral infection or sepsis [1]. Clinically, sepsis still has a high incidence and mortality, and remains a major public health problem worldwide. In 2017, the global number of new sepsis cases was estimated at 48.9 million (95% UI: 38.9–62.9), with an estimated 11 million sepsis-related deaths (95% UI: 10.1–12.0) [2]. The challenging development of existing therapies and their limited benefits underscore the pressing need for early diagnosis and targeted treatments. Among these, the cytokine storm is the most important characteristic of sepsis. It refers to an overwhelming shift toward pro-inflammatory responses over anti-inflammatory responses, resulting in systemic inflammatory amplification and immune homeostasis. High levels of serum ferritin were closely associated with increased formation of neutrophil extracellular traps (NETs) and aggravation of lung injury in sepsis patients, suggesting that ferritin might become a significant marker for assessing inflammation severity and immune imbalance. There is evidence that its level is closely associated with overall prognosis in sepsis patients [3].

Although therapeutic strategies for sepsis have greatly improved and may include antimicrobial therapy, fluid resuscitation, and supportive organ therapy in clinical practice, single-target intervention is not compatible with the complex supernetwork exhibited by the cytokine storm. The intercellular signaling pathways in sepsis represent a coordinated immunometabolic cross-talk. Studies have demonstrated that hepatocyte mitochondria are metabolically linked to TREM2-expressing liver macrophages, and disruption of this coordination may be further amplified during inflammatory responses and organ damage. This indicates that intercellular metabolic communication may represent a novel point of entry for precision interventions [4]. Additionally, GABA regulation, metabolic reprogramming, and the inflammatory macrophage response mediated by protein succinylation are also implicated, which complicates the non-linear dynamics of the pathophysiological mechanisms in sepsis. This complexity explains why the therapies developed so far have such narrow application ranges. Furthermore, the crosstalk within the inflammatory network, negative feedback regulation, and immune suppression in late-phase sepsis induce a transition from excessive inflammation to an immunodeficiency state, which predisposes patients to secondary infections and death. This also implies that early in the course of treatment, blood cultures probably have poor sensitivity for diagnosing patients with severe sepsis, especially if empirical antimicrobial therapy has been initiated [5].

In light of the above background, a systematic study of the multi-center network mechanisms in the sepsis cytokine storm, as well as immune regulation features and possible intervention strategies, has important theoretical and clinical implications. This review summarizes the main pathological mechanisms of sepsis, such as hyperimmune activation, the inflammatory cytokine network, cell communication disturbances in the microcirculation, metabolic reprogramming, and multiple modalities of cell death, and discusses immune regulation and negative feedback mechanisms that maintain host homeostasis. In addition, the review also discusses recent progress in precision medicine, biomarkers, and novel immunomodulators to establish a theoretical basis for multi-targeted and controllable intervention strategies.

2 MOLECULAR MECHANISMS OF THE CYTOKINE STORM

2.1 Overactivation and dysregulation of the immune system

The pathologic immune response in sepsis, commonly referred to as a "cytokine storm", reflects a profound imbalance between innate and adaptive immunity [6]. During early infection, macrophages, dendritic cells, and neutrophils are activated through pattern recognition receptors, including Toll-like receptors (TLRs), NOD-like receptors, and RIG-I-like helicases, leading to activation of central inflammatory signaling pathways such as NF-κB, MAPK, and JAK/STAT [7, 8]. Rather than reiterating their canonical cascades here, it should be noted that in sepsis these pathways become persistently amplified, driving excessive production of TNF-α, IL-1β, IL-6, IL-8, and IFN-γ and forming self-reinforcing inflammatory loops that culminate in tissue injury. The synergistic effect of TNF-α and IFN-γ can induce lethal cytokine shock, whereas inhibition of PANoptosis alleviates tissue damage and improves survival [8]. In addition, NONO enhances extracellular signal-regulated kinase (ERK)1/2 activation and cytokine release, while aberrant NLRP3 inflammasome activation serves as a pivotal amplification node contributing to sustained inflammation and multiple organ dysfunction syndrome (MODS) [6].

Excessive innate immune activation further promotes neutrophil extravasation and endothelial cell injury. Elevated ferritin can induce systemic inflammation in a macrophage scavenger receptor-dependent manner and promote NET formation through the coordinated activity of PAD4, neutrophil elastase, and reactive oxygen species, thereby aggravating acute lung injury (ALI) and microcirculatory dysfunction [3, 9]. Direct endothelial cell–neutrophil interaction also enhances NET generation, and the adhesion molecule macrophage-1 antigen (Mac-1) has emerged as a potential therapeutic target for mitigating NET-associated inflammation [9].

Adaptive immune dysfunction likewise contributes to both hyperinflammation and subsequent immune paralysis. The ATP–P2X purinoceptor 7 pathway regulates liver pannexin 1–IL-33 immune homeostasis, and exogenous IL-33 promotes $ST2^+$ regulatory T cell (Treg) expansion, reducing cytokine storm severity and improving survival in sepsis models. GPR174-deficient Tregs facilitate macrophage repolarization toward the anti-inflammatory M2 phenotype and suppress IL-6

and TNF-α production [10]. Conversely, IL-1R2$^+$ monocytes/macrophages generated by granulocyte-macrophage colony-stimulating factor (GM-CSF) and lipopolysaccharide (LPS) exhibit reduced HLA-DR expression and upregulation of immunosuppressive markers such as MS4A4A and CD63, representing a sepsis-like immunoparalytic phenotype [11].

Under chronic inflammatory conditions, the indoleamine 2,3-dioxygenase 1 (IDO1)-AHR- CYP1A1 axis forms a positive feedback loop with canonical cytokine signaling pathways, further amplifying inflammatory responses; this process can be attenuated by IDO1 inhibition [12]. Meanwhile, myeloid differentiation primary response 88 (MyD88)-dependent signaling in myeloid cells and cardiomyocytes contributes to endotoxin shock-induced systemic inflammation and cardiac dysfunction. Collectively, immune dysregulation in sepsis arises from integrated processes of pathogen sensing, signaling amplification, metabolic imbalance, and impaired immune regulation, leading not only to organ damage but also to both immune paralysis and secondary infections.

2.2 Inflammatory cytokine networks and signaling pathways

The cytokine storm is caused by the excessive release of several pro-inflammatory cytokines [13, 14]. A characteristic of this storm is the dynamic imbalance between the innate and adaptive immune responses, resulting in dysregulated systemic inflammation. In the context of sepsis and its accompanying ALI, the induction of this inflammatory cytokine network exhibits dynamic, non-linear amplification properties [13]. Several driving cytokines/chemokines such as TNF-α, IL-1β, IL-6, IFN-γ, IL-8, GM-CSF, and MCP-1 establish a positive feed-forward loop by cross-activating multiple signal transduction pathways, thereby aggravating tissue injury [13, 14].

Molecularly, the NF-κB, JAK/STAT, MAPK, and NLRP3 inflammasome pathways are the central nodes governing the cytokine storm [15]. In addition, Huashi Baidu Decoction inhibits the cytokine storm by modulating the TLR4/NF-κB and PI3K/Akt signaling pathways [13]. On the other hand, COB-187 suppresses inflammatory cascades by attenuating the DNA-binding activity of NF-κB (p65/p50) and the phosphorylation of IRF-3 at Ser396. Additionally, the JAK/STAT signaling pathway amplifies inflammation by regulating secondary cytokine production via IFN and IL-6 signaling. Persistent phosphorylation of STAT1/3 is associated with high-level inflammation in cells, whereas inhibitors of the JAK/STAT pathway inhibit IFN-α/β signaling and exert immunomodulatory effects in excessive inflammatory conditions such as macrophage activation syndrome [16].

Furthermore, the activation of MAPKs (p38, ERK, JNK) regulates inflammatory cytokine production and contributes to apoptosis, necrosis, and NET formation, thereby enhancing tissue injury. The NLRP3 inflammasome promotes caspase-1-mediated maturation of IL-1β and IL-18 and induces pyroptosis, further amplifying inflammation [17, 18]. For instance, luteolin and *Scutellaria baicalensis* Georgi and *Coptis chinensis* Franch ameliorate cytokine release syndrome by mediating the NLRP3/gasdermin D (GSDMD) pyroptosis pathway and its upstream CD39 purinergic signaling [17]. LPC, however, increases NLRP3 acetylation via the GITR-MARCH7-SIRT2 axis and promotes NLRP3 overactivation and macrophage pyroptosis, thereby worsening systemic inflammatory injury [18]. ACSS2 controls the activation of NLRP3 in renal tubular epithelial cells via the KLF5/NF-κB pathway, indicating that metabolic reprogramming is closely integrated with the inflammatory response [19].

In addition to these main pathways, cellular iron metabolism and ferritin also enhance inflammation. Studies have revealed that disruptions in iron homeostasis impair the ability of myeloid immune cells to regulate NF-κB and inflammasome activities, thus posing serious pathophysiological challenges for septic patients with iron overload [20]. Epidermal growth factor receptor (EGFR) has been confirmed as an important regulator of TNFR1-mediated inflammation and RIP3-dependent necroptosis, providing theoretical support for the potential immunological mechanisms of anti-EGFR therapy in sepsis-associated ALI [21].

Collectively, these interconnected signaling pathways form self-amplifying networks that drive excessive cytokine release and immune imbalance in sepsis. To provide a systematic overview of these mechanisms, this review summarizes key pro-inflammatory cytokines, their associated signaling pathways, and regulatory features involved in the cytokine storm in **Table 1**.

2.3 Cellular communication and inflammatory cascades

The septic cytokine storm is characterized by cell-to-cell communication, which is the driving force for systemic cascading inflammation. Communication between immune cells and endothelial cells is also key to this process. They form an intricate signaling network, connected by numerous means such as direct cellular contact, excretion of soluble factors, and extracellular vesicles (EVs), thus coordinating the initiation, amplification, and resolution of inflammation. Experiments have shown that direct interaction between bone marrow-derived mesenchymal cells and macrophages greatly enhances the production of IL-6, IL-10, and nitric oxide (NO). The generation of TNF-α is also suppressed by prostaglandin E_2. As a result, bone marrow-derived mesenchymal cells may alleviate systemic inflammation and modulate tissue inflammation, thereby maintaining immune homeostasis [46]. EVs carry MFG-E8, which facilitates the complete phagocytosis of apoptotic cells and therefore decreases systemic inflammation, as well as serving a protective function in sepsis [47]. In addition, bone

Table 1. Key inflammatory mediators and signaling pathways associated with cytokine storm

Inflammatory mediator	Major signaling pathway	Downstream targets	Function/role	References
TNF-α, IFN-γ	JAK/STAT1/IRF1 axis	Caspase-8	Induces PANoptosis-mediated inflammatory cell death	[8]
IL-6	Innate immune inflammatory signaling	Acute-phase reactants	Triggers systemic fatal inflammatory response	[22]
TNF-α, IL-6	Heparanase-dependent inflammatory signaling	Endothelial glycocalyx and adhesion molecules	Promotes inflammatory responses and organ injury	[23]
Ferritin	MSR/PAD4/ROS-dependent pathway	NETs	Induces systemic inflammation and lung injury	[3]
ACOD1 (IRG1)	CDK2→MAPK8→JUN axis	TNF signaling and pro-inflammatory cytokines	Activates cytokine storm and promotes inflammatory responses	[24]
TNF-α, IL-6	HIF-1α-dependent glycolysis	Macrophage metabolism and NF-κBp65 activity	Inhibits pro-inflammatory responses and cytokine storm	[25]
Pro-inflammatory M1 macrophage cytokines	NF-κB/ERK1/2 signaling pathway	M1 macrophage activity	Inhibits cytokine storm and ALI	[26]
TNF-α, IL-1, IL-6	LPS-induced inflammatory pathway	Serum inflammation levels	Regulates pro-inflammatory mediator expression	[27]
TNF-α, IL-6	Inflammation and oxidative stress pathways	Serum inflammatory cytokines, oxidative stress	Inhibits inflammatory cytokines and alleviates oxidative stress	[28]
Pro-inflammatory cytokines	P2Y2 receptor-mediated pathway	JNK, MMP-9, iNOS, NLRP3	Alleviates liver damage, suppresses inflammation, and improves survival	[29]
IL-1β/TNF-α	NF-κB pathway	GSDMD	Exacerbates alveolar epithelial injury	[30]
Various pro-inflammatory cytokines	IDO1-AHR-CYP1A1 axis	STAT3, NF-κB/STAT1, JNK/p38	Promotes cytokine storm and cell apoptosis	[12]
IL-1β, IL-18, IL-10	Caspase-11/GSDMD	GSDMD	NAD^+ blocks GSDMD-mediated pyroptosis and promotes immune homeostasis	[31]
TNF-α, IL-1β	PD-1 checkpoint	M1 marker (iNOS)	SMEP suppresses pro-inflammatory responses	[32]
TNF-α/IL-6	HIF-1α/LDHA	ICAM-1/VCAM-1/VEGF-A	Sepsis-induced endothelial injury	[33]
IL-1β, IL-18	NLRP3 inflammasome pathway	NETs	Promotes immunothrombosis and multi-organ injury	[34]
NLRP3	P2X7-NLRP3	Caspase-1	Mediates pyroptosis and inflammatory responses	[35]
cfDNA	Activation of TLRs/NF-κB pathway	NF-κB	Activates inflammatory responses	[36]
STAT3	IL-6/STAT3	FUNDC1	Regulates MAMs formation and mediates myocardial dysfunction	[37]
TNF-α, IL-1β	FAK-Pyk2, p38 MAPK pathway	NLRP3 inflammasome	Pro-inflammatory effect	[38]
IL-6, IL-1β, TNF-α	NLRP3 inflammasome	ROS/mtROS	Mitigation of cytokine storm	[39]
IL-6, IL-1β	ERK/JNK/NF-κB pathway	PGE_2	Induces PGE_2 production and participates in inflammatory responses	[40]
Tumor necrosis factor	TLR4	Caspase 3/7	Induces apoptosis	[41]
COX-2	NF-κB	PGE_2	Produces PGE_2 and mediates inflammatory responses	[42]
TNF-α	NF-κB/Akt pathway	$CD38^+$ macrophages	Regulates the cytokine storm in sepsis	[43]
IL-1β, IL-18	NF-κB/NLRP3 inflammasome	Caspase-1	Alleviates hepatic injury in septic mice	[44]
IL-1β	NLRP3 inflammasome	KLF2	Targets therapy for neonatal sepsis	[45]

Note: TNF-α, tumor necrosis factor-alpha; IFN-γ, interferon-gamma; JAK/STAT1/IRF1, Janus kinase/signal transducer and activator of transcription 1/interferon regulatory factor 1; IL-6, interleukin-6; MSR, macrophage scavenger receptor; PAD4, peptidyl arginine deiminase 4; ROS, reactive oxygen species; NETs, neutrophil extracellular traps; ACOD1, aconitate decarboxylase 1; IRG1, immunoresponsive gene 1; CDK2, cyclin-dependent kinase 2; MAPK8, mitogen-activated protein kinase 8; JUN, Jun proto-oncogene; HIF-1α, hypoxia-inducible factor 1-alpha;

NF-κBp65, nuclear factor kappa-B subunit p65; ERK1/2, extracellular signal-regulated kinase 1/2; ALI, acute lung injury; LPS, lipopolysaccharide; P2Y2, P2Y purinoceptor 2; JNK, c-Jun N-terminal kinase; MMP-9, matrix metalloproteinase-9; iNOS, inducible nitric oxide synthase; NLRP3, NLR family pyrin domain containing 3; GSDMD, gasdermin D; IDO1, indoleamine 2,3-dioxygenase 1; AHR, aryl hydrocarbon receptor; CYP1A1, cytochrome P450 family 1 subfamily A member 1; STAT3, signal transducer and activator of transcription 3; IL-18, interleukin-18; IL-10, interleukin-10; NAD^+, nicotinamide adenine dinucleotide; PD-1, programmed cell death protein 1; LDHA, lactate dehydrogenase A; ICAM-1, intercellular adhesion molecule 1; VCAM-1, vascular cell adhesion molecule 1; VEGF-A, vascular endothelial growth factor A; P2X7, P2X purinoceptor 7; cfDNA, cell-free deoxyribonucleic acid; TLRs, Toll-like receptors; FUNDC1, FUN14 domain containing 1; MAMs, mitochondria-associated endoplasmic reticulum membranes; FAK, focal adhesion kinase; Pyk2, proline-rich tyrosine kinase 2; p38 MAPK, p38 mitogen-activated protein kinase; mtROS, mitochondrial reactive oxygen species; PGE_2, prostaglandin E_2; TLR4, Toll-like receptor 4; COX-2, cyclooxygenase-2; Akt, protein kinase B; CD38, cluster of differentiation 38; KLF2, Krüppel-like factor 2; SMEP, sepsis-modified extracellular peptides.

marrow-derived mesenchymal stem cell-derived exosomes induce M2 macrophage polarization by targeting the miR-20a-5p/CXCL12 axis, thereby effectively inhibiting the septic cascade [48].

By recruiting immune cells and regulating inflammation, Meteorin-like protein can direct the migration of macrophages, which in turn dramatically increases the body's resistance to sepsis, even as it balances new controls over Treg/Th17 immunity [49]. However, via the Snail/CXCL2 axis, PAK1 can enhance the inflammatory cascade following sepsis [50]. It is worth noting that in both infectious and noninfectious systemic inflammation, IL-6 can promote TNF-α production through the NF-κB pathway rather than classical STAT3 signaling, thus making inflammatory responses more severe [51].

In sepsis, there exists a highly complex and tightly coupled intracellular signaling network. Through multiple pathways within these networks, the inflammatory cascade can spread rapidly. The mechanisms by which innate immune activation and inflammatory cell death contribute to sepsis are dissected in this review by summarizing the classical and non-classical activation processes of the inflammasome signaling pathway in **Figure 1**. Via the TLR-NF-κB pathway, pathogen-associated molecular patterns induce the expression of pro-IL-1β and NLRP3. LPS can activate non-classical pathways mediated by caspase-11; this in turn leads to formation of the NLRP3-apoptosis-associated speck-like protein containing a CARD (ASC)-caspase-1 complex, in which GSDMD is cleaved and N-terminal pores are formed. This can induce pyroptosis and promote the maturation and release of IL-1β, thereby amplifying dysregulated inflammation and aggravating organ dysfunction during sepsis. As the understanding of these communication and signaling networks becomes more profound, the etiology of the cytokine storm is revealed, providing both a theoretical basis and a strategy for precise anti-inflammatory intervention by regulating major pathways or EV release.

3 PATHOPHYSIOLOGICAL MECHANISMS OF SEPSIS

3.1 Microcirculatory dysfunction and organ failure

Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection. Microcirculatory failure represents one of the key pathophysiological mechanisms underlying organ dysfunction in sepsis. This non-physiological state not only fails to provide acceptable tissue perfusion but also exacerbates the course of multiorgan failure [52, 53]. In sepsis, elevated PCSK9 levels can activate the TLR4/MyD88/NF-κB and NLRP3 pathways, thereby promoting dysregulated systemic inflammation and endothelial injury that contribute to organ dysfunction [54]. Concurrently, endothelial cell activation, upregulation of adhesion molecules, and increased vascular permeability enhance plasma extravasation and tissue hypoperfusion. Increased expression of Mac-1 on the neutrophil surface induces pulmonary microcirculatory dead space, and inhibitors of Mac-1 can partially improve ALI in sepsis, suggesting that microcirculatory perfusion abnormality is an important feature during organ dysfunction [55].

Microthrombosis is also a significant cause of microvascular dysfunction in sepsis. TNF-α, IL-1β, and HMGB1-associated proinflammatory signals promote both platelet aggregation and fibrin deposition, leading to subsequent capillary occlusion and non-uniform blood flow. Furthermore, high PAI-1 levels represent a disseminated intravascular coagulation state with suppressed fibrinolysis and are strongly related to organ dysfunction [53]. Reduced erythrocyte deformability and reduced blood flow worsen tissue hypoxia, such that microcirculatory perfusion may be impaired despite normal blood flow in the larger vessels, leading to a mismatch between perfusion and oxygenation even when large vessel blood flow is unimpaired [52]. Both clinical and experimental studies have demonstrated that septic shock is associated with peripheral vascular hyporesponsiveness, although some capillary beds within the post-capillary circulation retain residual responsiveness [56]. These findings imply that vasomotor regulatory function is an important factor in sustaining microcirculatory integrity.

Some interventions targeting microcirculatory failure have beneficial effects. For instance, Shenfu Injection enhances microcirculatory perfusion and endothelial function through suppression of PI3K/Akt-induced glycolysis [57]. Salvianolic acid B, meanwhile, mitigates microcirculatory failure and sepsis progression by suppressing platelet CD226 molecule function [58]. Multiple factors, including endothelial damage, an overwhelming inflammatory response, microthrombosis,

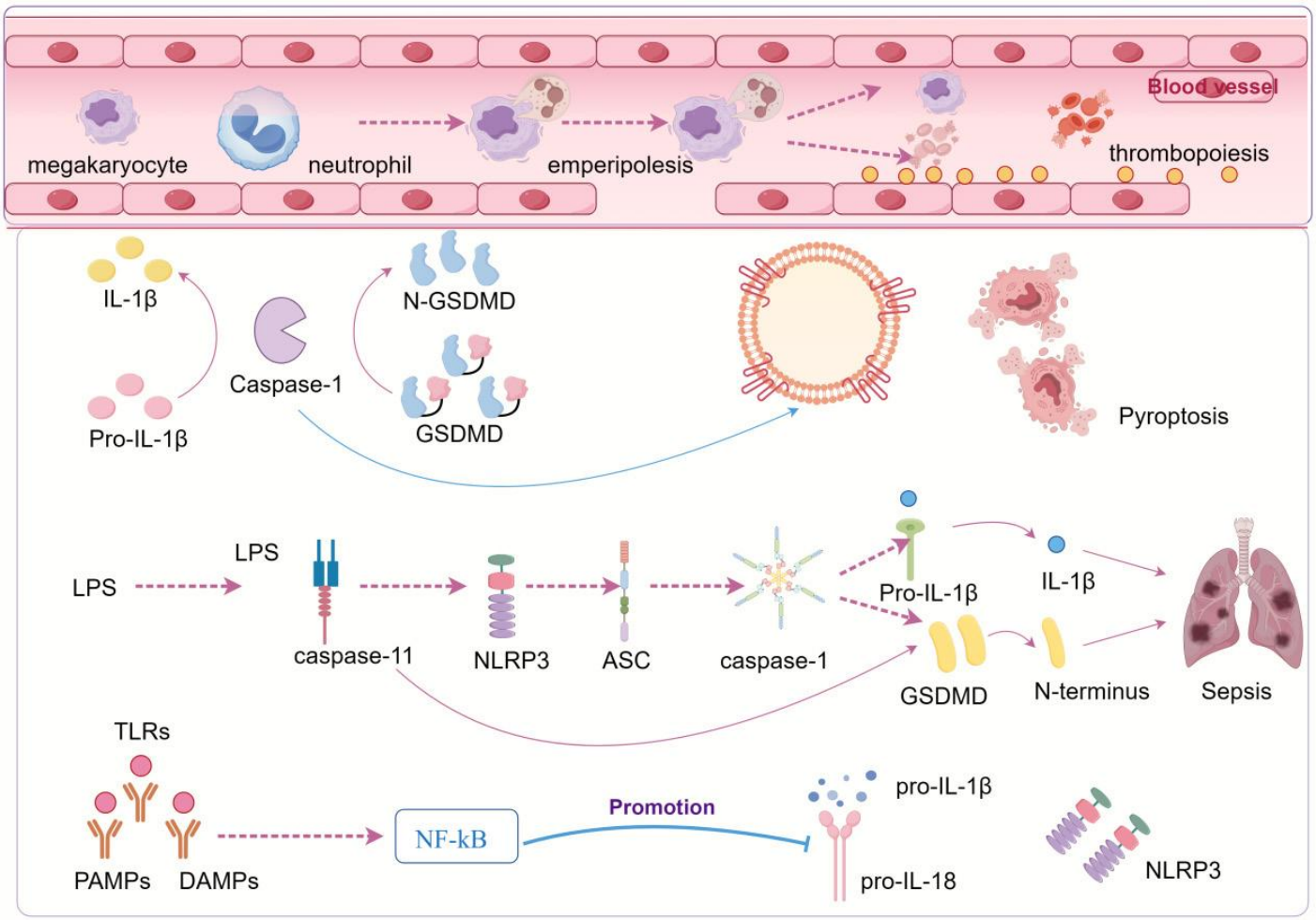

Figure 1. Mechanistic diagram of inflammasome activation and pyroptosis in sepsis and thrombopoiesis. This diagram illustrates how inflammasome activation contributes to excessive inflammatory responses and subsequent organ dysfunction during sepsis, as well as to platelet formation via GSDMD-driven pyroptosis. TLRs recognize PAMPs and DAMPs and then activate NF-κB signaling, prompting the expression of pro-inflammatory cytokines such as pro-IL-1β and pro-IL-18, as well as NLRP3. Activation of caspase-11 by LPS contributes to NLRP3 inflammasome assembly, and caspase-1 is also activated at the plasma membrane via the adaptor protein ASC. N-GSDMD can form membrane pores, thereby enabling pyroptosis as well as the release of IL-1β. Thus, in sepsis model mice, this process accelerates both systemic inflammation and disease progression. The inset graph illustrates emperipolesis occuring between neutrophils and megakaryocytes. It suggests that this specific cellular response leads to both enhanced platelet formation and amplification of inflammation in the local vascular environment.

and imbalanced oxygen delivery, play a role in the pathogenesis of septic microcirculatory dysfunction. These mechanisms interact to generate a complex network, resulting in multiorgan failure. This explains the clinical approach toward multitarget interventions (microcirculation protection, anti-inflammatory treatment, and antithrombosis therapy).

3.2 Pathogen recognition and host response

Sepsis is a severe syndrome caused by microbial infection and characterized by excessive host inflammatory responses and organ injury [59]. In this process, pathogen-associated molecular patterns and damage-associated molecular patterns are recognized by pattern recognition receptors expressed on epithelial and endothelial cells of barrier tissues, as well as on circulating and resident innate immune cells, thereby initiating early innate immune responses. This represents a generalized mechanism of early host–pathogen interaction in sepsis rather than a process restricted to a specific anatomical site or experimental model, thereby triggering the initial immune defense [60].

Neutrophils assemble inflammasome complexes in distinct subcellular compartments and, in sepsis-relevant models, predominantly release mature IL-1β and IL-18. In these settings, extracellular IL-1α and IL-33 are often minimal or undetectable, indicating a context-dependent and selective inflammasome-associated cytokine output rather than a universal paradigm. This suggests that the pro-inflammatory function of the inflammasome during sepsis is highly selective [60].

Table 2. Pathophysiological mechanisms and clinical manifestations associated with sepsis

Inflammatory cytokines	Key signaling pathways	Downstream targets	Functions/effects	References
NLRP3 inflammasome	Calcium influx-mediated pathway	Caspase-1	Exacerbates inflammatory responses in sepsis	[72]
TNF-α	NF-κB	NLRP3	Induces macrophage pyroptosis associated with sepsis	[73]
HMGB1	RAGE–LPS internalization pathway	Caspase-11	Mediates pyroptosis and sepsis-induced lethality	[74]
IL-1β	NLRP3 inflammasome pathway	NF-κB	Leads to sepsis-associated myocardial atrophy and contractile dysfunction	[75]
TNF-α, IL-6	CD14/Syk/PLCγ2 pathway	MyD88	Excessive inflammation contributes to the development of sepsis	[76]
IL-1β	Caspase-11-GSDMD pathway	NLRP3 inflammasome	Mediates pyroptosis and septic shock	[77]
miR-21	ADAR1/miR-21/A20 axis	NLRP3 inflammasome	Inhibits macrophage pyroptosis induced by sepsis	[78]
TNF-α	TAK1/NF-κB/MAPKs, Nrf2 pathways	TAK1, Keap1	Ameliorates sepsis	[79]
IL-6	gp130–JAK2–STAT3 pathway	SOCS3 gene expression	Mediates muscle atrophy in sepsis	[80]
IL-1β	Pyroptosis pathway	GSDMD	NSA inhibits pyroptosis to alleviate sepsis	[81]
IL-1β/IL-6/TNF-α	Notch1–NF-κB pathway	Tight junction proteins	Regulates sepsis-associated gut–brain axis inflammation	[82]
IL-6	IL-6/STAT3 signaling pathway	STAT3	Suppresses inflammation and apoptosis in sepsis	[83]
GDF15	AMPK/NF-κB/MAPK pathways	eIF2α/ATF4 transcription axis	Inhibits sepsis-associated inflammation and glycolysis	[84]
IL-1β	NLRP3 inflammasome pathway	Tissue factor	Promotes sepsis-associated coagulation and thrombosis	[85]
TNF-α/IL-6	TLR4 signaling pathway	LC3-II/p62	Promotes anti-inflammatory responses and activates autophagy during sepsis	[86]
IL-1β	ADRA2B-cAMP-PKA pathway	GSDMD cleavage	Inhibits sepsis-associated pyroptosis	[87]
Citrullinated histone H3	TLR2-Ca^{2+}-PAD2 axis	PAD2 auto-citrullination and nuclear translocation	Promotes sepsis-associated NETs pyroptosis	[88]
IL-1β	NLRP3 inflammasome pathway	Mitochondrial ROS and autophagy	Inhibits sepsis-associated inflammasome activation	[89]
TNF-α/IL-6/IL-1β	STAT3/NF-κB/NLRP3 pathways	GPR18	Alleviates ALI induced by sepsis	[90]
IL-1β/IL-6/TNF-α/MCP-1	miR-122-5p–HO-1/MASP1 pathway	MASP1/HO-1	Regulates sepsis-associated coagulation and inflammatory responses	[91]
TNF-α/IL-6/IL-1β	TLR4/MAPK/NF-κB pathway	TLR4–MD2 complex	Inhibits the production of sepsis-associated inflammatory cytokines	[92]
TNF-α, IL-6	NF-κB pathway	VCAM-1/ICAM-1	Attenuates sepsis-induced ALI	[93]
IL-1β	NLRP3 inflammasome pathway	NLRP3, caspase-1	Inhibits sepsis-associated pyroptosis	[94]
TNF-α/IL-1β	NF-κB pathway	TRPM7	Reverses LPS-induced septic cardiomyocyte injury	[95]
GPIbα cytoplasmic tail–mediated regulation of inflammatory cytokines	PKC-dependent pathway	GPIbα cytoplasmic tail	Exacerbates sepsis-associated platelet activation and inflammation	[96]
TNF-α, IL-6, IL-1β	Ubiquitination–lactylation pathway	CDT2–KAT2A axis	Ameliorates myocardial injury in septic cardiomyopathy	[97]
IL-1β, IL-6, TNF-α	NF-κB inflammatory pathway	NFKBIA mRNA	Alleviates neuroinflammation in sepsis-associated encephalopathy	[98]

Note: NLRP3, NLR family pyrin domain containing 3; TNF-α, tumor necrosis factor-alpha; NF-κB, nuclear factor kappa-B; HMGB1, high mobility group box 1; RAGE, receptor for advanced glycation end products; LPS, lipopolysaccharide; IL-1β, interleukin-1 beta; IL-6, interleukin-6; CD14, cluster of differentiation 14; Syk, spleen tyrosine kinase; PLCγ2, phospholipase C gamma 2; MyD88, myeloid differentiation primary response 88; GSDMD, gasdermin D; miR-21, microRNA-21; ADAR1, adenosine deaminase acting on RNA 1; A20, tumor necrosis factor alpha-induced protein 3 (TNFAIP3); TAK1, transforming growth factor-beta-activated kinase 1; MAPKs, mitogen-activated protein kinases; Nrf2, nuclear factor erythroid 2-related factor 2; Keap1, Kelch-like ECH-associated protein 1; gp130, glycoprotein 130; JAK2, Janus kinase 2; STAT3, signal transducer and activator of transcription 3; SOCS3, suppressor of cytokine signaling 3; NSA, necrosulfonamide; Notch1, neurogenic locus notch homolog protein 1; GDF15, growth differentiation factor 15; AMPK, AMP-activated protein kinase; eIF2α, eukaryotic initiation factor 2 alpha; ATF4, activating transcription factor 4; TLR4, Toll-like receptor 4; LC3-II, microtubule-associated protein 1 light chain 3-II; p62, sequestosome-1; ADRA2B, adrenoceptor alpha 2B; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; PAD2, peptidyl arginine deiminase 2; ROS, reactive oxygen species; GPR18, G protein-coupled receptor 18; ALI, acute lung injury; MCP-1, monocyte chemoattractant protein-1; HO-1, heme oxygenase-1; MASP1, mannan-binding lectin serine protease 1; MD2, myeloid differentiation factor 2; VCAM-1, vascular cell adhesion molecule 1; ICAM-1, intercellular adhesion molecule 1; TRPM7, transient receptor potential melastatin 7; GPIbα, glycoprotein Ib alpha chain; PKC, protein kinase C; CDT2, full name to be confirmed, a RING-type E3 ubiquitin ligase; KAT2A, lysine acetyltransferase 2A; NFKBIA, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor alpha.

In monocytes, TLR agonists induce an overall similar pattern of glycoprotein expression changes, but the profile changes under *Staphylococcus aureus* stimulation is partially distinct. Eleven glycoproteins (CD44, CD274, LILRB1, ICAM1, DSC2, PTGS2, LAMP3, CR1, FGL2, HP, and SLC1A3) are consistently dysregulated in the tolerant cell state [61].

C-ter100 can activate NF-κB through TLR4 and promote TNF-α and NO generation, as well as provoke NLRP3 inflammasome assembly (NLRP3, ASC, caspase-1) and IL-1β secretion [62]. Loss of nucleic acid recognition is likely to contribute to the lack of early detection of *S. pyogenes*, diminishing local infection control and ultimately initiating systemic inflammation at later stages [63]. Expression and regulation of monocyte TLR-2 may be abnormal in septic patients, whereas those for TLR-4 are relatively low [64].

Peritonitis can increase expression of peritoneal exudate cells genes involved in sepsis but does not augment this response after LPS pretreatment [65]. FLAP and 5-LO-dependent eicosanoids are also involved in bacterial endotoxin-induced inflammation through TLR-dependent mechanisms [66]. Genetic background, gut microbiota composition, and metabolic status modulate the magnitude and kinetics of the host immune response. Together, these components of the ambivalent inflammatory response in sepsis dictate whether pathogen elimination through inflammation, or instead, characteristic hyperinflammation will prevail.

3.3 Metabolic reprogramming and cell death

Metabolic reprogramming and pyroptosis during sepsis progression are mutually intertwined, propagating the inflammatory response and ultimately resulting in multiorgan failure. It has been reported that these interventions, including scavenging of lipid peroxidation by the antioxidant vitamin E, chemical inhibition of PLCG1, and gene deletion for Caspase-11/GSDMD, could significantly modulate ferroptosis and other metabolism-dependent cell death pathways and ameliorate tissue damage induced by polymicrobial sepsis [67].

EGFR mediates the membrane translocation of Glut1 via the downstream TBK1/Exo84/RalA protein pathway, which results in Warburg effect. This reinforces the activation and apoptosis of $CD4^+$ T lymphocytes, further resulting in damage to immune function and contributing to accelerated immunological exhaustion. Furthermore, IL-1R2 physically interacts with enolase 1, which can suppress glycolysis-mediated pyroptosis and inflammation, thus indicating a therapeutic target within this pathway. Dexmedetomidine can also help maintain metabolic balance by upregulating Nrf2, inhibiting mitochondrial fission, and downregulating ferroptosis and vascular leakage [68]. Meanwhile, aerobic glycolysis suppression can activate autophagy via the lactate/SIRT3/AMPK signaling pathway, thereby providing protection in sepsis-associated acute kidney injury (AKI) [69].

Mechanistically, ferroptosis and lipophagy contribute significantly to septic AKI, which has added new insight into the pathogenetic mechanisms and therapeutic targets of AKI [70]. Moreover, knockout or knockdown of MAPL also attenuates septic myocardial injury and inflammation by inhibiting Drp1 SUMOylation and ameliorating mitochondrial dysfunction. These findings suggest that metabolic reprogramming and multimodal cell death are tightly interconnected in sepsis and together contribute to a dysregulated host response to infection, characterized by excessive inflammation followed by immune suppression, which ultimately results in life-threatening organ dysfunctions [71].

Energy metabolism pathways can be regulated, selected forms of cell death can be inhibited, and autophagy can be stimulated, resulting in attenuation of the organ injury response and systemic inflammation. To systematically reveal the pathological processes and clinical manifestations critically associated with sepsis development, priority mechanisms are summarized in **Table 2**. The pathophysiology of sepsis is complex and multifaceted, comprising interactions among immune dysfunction, microcirculatory derangement, metabolic disarray, and cell death networks. These mechanisms collectively contribute to the progression from localized infection to dysregulated sys-

temic host response and subsequent organ dysfunction: microcirculatory dysfunction induces tissue hypoxia; abnormal recognition and/or excessive response to pathogens will excessively activate the immune system; and metabolic disorders activated by these former processes, along with programmed cell death itself, further enhance inflammation-induced injury. This mechanistic framework informs the pathogenesis of sepsis and identifies core nodes with their intrinsic relationships at different levels, from molecule to system, thus offering theoretical support for early identification and multi-target combination interventions.

4 REGULATION AND DYSREGULATION OF IMMUNE RESPONSES

4.1 Immunosuppressive phase and immune evasion

Studies have demonstrated that the immunosuppressive phase of sepsis involves sophisticated immune regulation and evasion, which are mediated through broad interplays among cellular functions, metabolism, and signaling. Neutrophils with high expression of CD200 receptor may also induce systemic immunosuppression by promoting Treg cells [99]. Indeed, recent investigations also indicate that metabolic reprogramming acts as a pivotal mediator of crosstalk between immune cell subsets in sepsis. Inflammatory stress and hypoxia direct immune cells into a state of increased glycolysis and modified lipid metabolism, resulting in remodeling of immune differentiation and functional polarization. Metabolic rewiring, for instance, skews T-cell subsets toward regulatory phenotypes, and lactate accumulation inhibits effector T-cell responses. Macrophage polarization is tightly associated with metabolic state. These results suggest that metabolic changes actively drive immune subset dysregulation during sepsis and are not simply a reflection of immune activation [100]. This indicates that intracellular metabolic regulation could be a novel therapeutic approach for sepsis-induced immunosuppression [101]. $CXCR2^+$ neutrophil subtypes also participate in immune suppression, which may warrant their consideration as therapeutic targets [102].

TCF7 and LEF-1 overexpression can significantly upregulate the proliferation capability and effector function of $CD4^+$ T cells, inhibit sepsis-induced apoptosis, and downregulate the expression of PD-1/LAG-3, resulting in a stronger immune response in patients with sepsis [103]. Metabolic dysfunctions have an intrinsic role in immunosuppression: lactate impairs T cell activation by downregulating CD40LG and SOCS3 expression, consequently restraining the JAK-STAT signaling pathway [104]. Low-density neutrophils suppress T cells through PD-L1 and contribute to susceptibility to secondary infections [105]. Neutrophil immune function can be compromised by glycolysis blockage, which downregulates lactate dehydrogenase A via the PI3K/Akt-HIF-1α signaling pathway [106]. The Spns2/S1P signaling pathway is important for regulating immune homeostasis, inhibiting excessive early inflammation, and relieving delayed immunosuppression, which may have a critical role in immune remodeling [107].

These investigations systematically demonstrate the hallmarks of regulatory networks in the immunosuppressive process of sepsis and underscore that the generation of Treg cells, subset properties of neutrophils, and dysregulated cellular metabolism are crucial hubs for immune evasion. They support rational drug discovery targeting metabolic reprogramming, immune checkpoint regulation, and signaling pathways.

4.2 Negative feedback mechanisms and host homeostasis

Targeted deletion of IL-10 in $CD169^+$ macrophages results in dramatically increased mortality during septic challenge, and administration of recombinant IL-10 is protective in a model of LPS-induced lethality, thereby highlighting the importance of anti-inflammatory mediators during early negative feedback regulation of inflammation [108, 109]. Adenosine deaminase acting on RNA 1 (ADAR1) inhibits sepsis-related ALI by suppressing the activation of pyroptosis in pulmonary macrophages via the miR-21/A20/NLRP3 axis, demonstrating that the host provides a possible negative feedback-based control mechanism to prevent inflammation propagation and tissue damage at the molecular level [78]. In patients with sepsis, T cell immunoreceptor with Ig and ITIM domains $(TIGIT)^+$ T cells display PD-1 upregulation, CD226 downregulation, and impaired cytokine secretion, remarkably, in vitro blocking of TIGIT restores T cell activity. These results imply that the inhibitory receptor modulates T cell activity through negative signaling to balance the immune response and avoid excessive inflammation [110].

The IL-10/DEL-1 axis not only favors emergency granulopoiesis over neutropenia but also promotes early host survival by balancing the abundance and function of immune cells, thus emphasizing the role of immune negative feedback in inducing overall immune homeostasis [108, 109, 111]. In addition, the finding that the absence of IL-10 in B cells leads to aberrant cGMP-PKG signaling, and that exogenous IL-10 supplementation alleviates LPS-induced ALI, suggests that negative feedback loops maintain both local and systemic immune homeostasis through dense network interactions with cytokines and immune cells [111]. In CLP-induced sepsis survivor mice, splenic $CD11b^+$ $Ly6C^{high}$ myeloid cells are significantly expanded at 4 weeks post-injury, and are predominantly composed of monocytic myeloid-derived suppressor cells with evident metabolic reprogramming. Overall, this population exhibits M-MDSC–like metabolic features, suggesting that immunosuppressive myeloid cells participate in post-inflammatory immune regulation after sepsis in a dynamic and adaptive manner [112].

IL-4 induces LAMP2 expression by activating STAT3 in lysosomal homeostasis and autophagic flux, and this metabolic and cellular homeostatic regulation is also involved in negative feedback control [113]. Furthermore, artemisinin-based therapy rescues both inflammatory and immunosuppressive states in sepsis immune cells, therefore globally restoring the homeostasis of the host's immunological defense function, suggesting that these negative feedback mechanisms play an integrative regulatory role in controlling host defense.

4.3 Role of immunoregulatory cells

During the development of sepsis and the cytokine storm, immunoregulatory cells are critically involved in the regulation of host immune homeostasis and the downregulation of excessive inflammation. They inhibit hyperactivation of effector T cells, dendritic cells, and other immune-activated cells through the growth factors IL-10 and TGF-β, as well as through contact-mediated inhibitory mechanisms, thus locally restricting inflammation to control tissue damage. Production of reactive oxygen species and NO by MDSCs is the most common mechanism to inhibit T cell proliferation, as well as to control amino acid metabolism pathways and the inflammatory activity of macrophages and neutrophils. At the height of inflammation, the explosive accumulation of MDSCs acts to protect tissues from injury, while their protracted survival can contribute to immunosuppression.

Local immune self-regulation, however, is still modulated by tissue-resident regulatory macrophages and dendritic cell subtypes that not only produce anti-inflammatory/proresolving mediators and phagocytose apoptotic cells but also regulate the expression of co-stimulatory molecules to dampen hyperresponsiveness, thereby essentially inhibiting overt inflammatory responses. At the molecular level, Nrf2 plays a role in protecting against sepsis-induced lung injury by modulating autophagy and NF-κB/PPARγ-dependent macrophage polarization [114]. GPR174 affects early immune regulation in sepsis by altering macrophage phenotype and the production of both pro- and anti-inflammatory cytokines [10]. In addition, the 'mature DCs enriched in immunoregulatory molecules' program is activated within 24 hours after sepsis onset via the TNFRSF-NF-κB and IFNGR2-JAK-STAT3 pathways, highlighting the critical role of dendritic cells in early immune modulation [115].

PAD2/PAD4 deletion restrains NLRP3 activation and accelerates the resolution of inflammation by skewing the $Ym1^+$ M2 macrophage phenotype toward a resolving phenotype in the lungs. Thus, the PADIs/NLRP3/Ym1 axis may be a novel therapeutic target for sepsis-associated ALI [116]. Repeated sepsis worsens $CD4^+$ T cell exhaustion and the antiviral immune response, leading to poor outcomes; administration of anti-TIGIT monoclonal antibodies can reverse T cell apoptosis caused by sepsis and significantly improve survival [117, 118]. Overall, dynamic regulation of Tregs, MDSCs, regulatory macrophages, and dendritic cells, combined with the factors involved in these key signaling pathways, constitutes a complicated immunoregulatory network in sepsis and provides an excellent theoretical basis for precision intervention and targeted treatment.

5 INNOVATIVE INTERVENTIONAL STRATEGIES

5.1 Precision medicine and personalized therapy

BAM15 promotes mitochondrial DNA-dependent responses and may serve as a potential companion biomarker for the initial diagnosis and efficacy monitoring of septic patients during therapy [119]. The immune response in sepsis is highly variable, and this heterogeneity has a profound impact on disease outcome and its treatment. Integrated analysis of patients' immune phenotypes, metabolic status, microbiome information, and genetic content leads to accurate stratification of the intensity and type of inflammatory responses and provides a solid scientific foundation for personalized interventions [120]. A typical application scenario may involve the patient population with a low resistance program molecular fingerprint compared with systemic inflammation levels, for which greater diversity of immune states is relevant for developing more sophisticated stratification strategies and adjustment of therapeutic schedules.

Certain combinations of IFN-γ and IL-1β can efficiently differentiate cytokine release syndrome from sepsis, thus significantly enhancing the accuracy of individualized therapeutic interventions [121]. Neutrophil-derived C1q has been proposed as a consistent prognostic biomarker for sepsis-associated death and could be a therapeutic target [122]. Furthermore, the transfer of apoptotic cells to patients with mild-to-moderate sepsis has been found to be safe and feasible, capable of directing immune responses and contributing to early termination of the cytokine storm [123]. As an essential autophagy-regulatory molecule, RAS protein activator-like 3 not only plays a role in the treatment of sepsis but also provides a new therapeutic target for other inflammatory conditions [124].

Using precise risk factors, such as the Acute Physiology and Chronic Health Evaluation III score, bicarbonate levels, anion gap, and invasive/noninvasive systolic blood pressure in combination, more personalized therapies can be tailored to improve survival time and prognostic outcomes in patients with sepsis-induced acute respiratory distress syndrome [125]. Furthermore, the heterogeneity of sepsis subtypes and differences in mortality among populations have been observed; such a tri-variable model can effectively identify patients with the δ sepsis subtype and thus form a reliable basis for dynamic monitoring and stratified intervention [126]. Integration of high-throughput multi-omics techniques, single-cell transcriptomic profiling, and AI algorithms allows real-time monitoring

of immune cell subsets and inflammatory networks. This holistic approach can offer an evidence-based strategy for appropriate drug choice, medication dosage, and timing of intervention in the cytokine storm to maximize its control, reconstitute immune balance, and ultimately improve patient outcomes.

5.2 Application of biomarkers in clinical decision-making

In independently predicting mortality among septic patients, lactate performs better than the quick sequential organ failure assessment (SOFA) score and equally well as the full SOFA score [127]. To improve translational clarity, the discussed biomarkers are interpreted within a phenotype-oriented framework. Sepsis-associated cytokine storms may manifest as hyperinflammatory, immunosuppressive, endothelial/organ dysfunction-dominant, or hypoperfusion/metabolic phenotypes. Rather than being viewed as isolated indicators, biomarkers should therefore be contextualized within these immune states to guide risk stratification and therapeutic prioritization [128]. EV-derived miRNAs may help differentiate sepsis from non-septic shock, and a three-miRNA composite can substantially enhance diagnostic potential, serving as an ideal decision-making tool for early intervention in this critical group of postoperative subjects [129]. The combination of serum CD64 and pro-adrenomedullin with the SOFA score has good discriminatory capability in patients with septic shock. Of these markers, CD64 is easier to detect and more convenient; it can partly replace complicated scores [130]. The Systemic Immune-Inflammation Index and procalcitonin have both demonstrated potential value in the prognostic assessment of sepsis, and their combined application may further improve the predictive accuracy for adverse outcomes in septic shock. However, current findings remain heterogeneous, and their clinical utility still requires further validation [131]. Early increased PTX3 levels in severe sepsis and septic shock are associated with the development of new organ dysfunction, while a smaller decrease in circulating PTX3 levels is associated with an unfavorable prognosis. Interestingly, in patients with septic shock undergoing albumin resuscitation, PTX3 levels are lower than in those receiving crystalloids [132]. BMP9 has been identified as a stratification biomarker with independent prognostic value, serving as a potential host-targeting strategy for sepsis treatment [133]. Evidence indicates that time-restricted feeding plays a hepatoprotective role in sepsis-induced hepatic injury, and 3-HB might represent a novel pharmacological target, and even a serum marker, for the treatment of severe liver injury of unknown cause, which could provide new ideas for the clinical treatment and risk assessment of hepatic injury [134]. Serum CXCL5 levels have also been suggested as a useful biomarker for enhancing sepsis diagnosis and outcome prediction, and can therefore be used in multivariate predictive models for better risk stratification and to guide treatment initiation [135].

However, no single biomarker can fully capture the complexity of the inflammatory network in sepsis, further supporting the need for phenotype-oriented multimarker integration. Multimarker composite features—such as lactate, cytokine profiles, immune cell functions, and metabolism—can offer a more accurate determination of patients' inflammatory status and immune competence for early intervention and personalized therapy. Continuous monitoring of important inflammatory mediators and immune parameters allows closer guidance of therapy with anti-infective and immunomodulatory drugs in real time, optimizing therapy duration and avoiding over-immunosuppression or overactivation. In an era of progressive development in single-cell analysis, metabolomics, and AI-driven predictive models, the clinical value of these biomarkers is anticipated to achieve a higher level of precision and personalization, providing firm support for accurate patient stratification and identification, tailored interventions, and improved outcomes in sepsis, and ultimately reducing sepsis-associated mortality.

5.3 Novel immunomodulators and anti-inflammatory agents

At the same time, the translational relevance of immunomodulatory spectra in sepsis becomes more substantive when treatment is specifically targeted to biologically delineated immune endotypes. Interventions such as adjunctive corticosteroids, rather than being applied uniformly, may be more properly considered within gene-expression-informed inflammatory states, which can identify patients likely to benefit most and mitigate the potential harms of indiscriminate immune suppression [136]. Sepsis and its attendant cytokine storm represent a condition of intricate immunopathobiology, characterized by marked dysfunction and dysregulation of the host immune response, including excessive activation of inflammation in conjunction with immunosuppression and multiple organ failure. In the context of increasingly deeper integration of omics technologies and AI, the therapeutic concept of sepsis has also been transforming from traditional anti-infection or supportive care to one involving precision stratification, dynamic monitoring, and multidimensional intervention. The multi-targeted regulation modes of immune disturbance, metabolic dysfunction, and inflammatory overreactions present challenges for patient therapy. In summary, to systematically summarize recent developments and prospects in sepsis treatment, this review addresses emerging interventional strategies reported in the past decade in **Table 3**, including precision medicine, biomarker-guided management, and immunomodulatory therapy, which may provide theoretical support for a personalized medicine-oriented individualized therapeutic approach as well as combined multi-target interventions.

In addition, melittin was demonstrated to relieve sepsis-induced AKI by suppressing ferroptosis mediated by the GPX4/NRF2 signaling [159]. GDF15 protects against sepsis-induced pulmonary injury by activating AMPK, inhibiting glycolysis, and inactivating the NF-κB/MAPK signaling pathways, leading to

Table 3. Innovative intervention strategies and application prospects

Intervention strategy	Target/mechanism	Research stage	Clinical/experimental outcomes	References
TaoHe ChengQi decoction	Nrf2-mediated anti-ferroptosis pathway	Validated in animal models	Improved sepsis-induced cardiac dysfunction	[137]
NLRP3 gene knockout	NLRP3/IL-1β signaling axis	Mechanistic validation in animal models	Ameliorated sepsis-induced myocardial atrophy	[75]
EphA4-Fc decoy receptor	Blockade of Eph/Ephrin signaling	Validated in animal models	Attenuated vascular leakage and endothelial dysfunction in septic mice	[138]
Biomimetic nanoregulator	Regulation of the Nrf2/HO-1 pathway	Validated in animal models	Improved cognitive function in sepsis-associated encephalopathy	[139]
Xuebijing	NF-κB pathway	Experimental validation stage	Reduced IL-1β expression levels in patients with sepsis	[140]
UDCA	Inhibition of the STING–PANoptosis axis	Validated in animal models	Alleviated sepsis-induced pulmonary edema and inflammation	[141]
FRC-derived exosomes	CD5L–PINK1/Parkin axis	Validated in mouse models	Improved renal function and increased survival rate in sepsis	[142]
CXCR1/2 inhibition	NETosis–CXCR1/2 axis	Validated in mouse models	Reduced multiorgan injury and mortality in sepsis	[143]
miR-223 macrophage therapy	Inhibition of glycolysis and maintenance of an anti-inflammatory phenotype	Validated in mouse models	Alleviated the severity of sepsis	[144]
MALAT1–STAT3 axis inhibition	Degradation of MALAT1 and p-STAT3	Validated in mouse models	Improved survival in septic mice	[145]
Palmatine	Notch1/NF-κB pathway	Validated in mouse models	Improved neurological function in mice with sepsis-associated encephalopathy	[82]
MON targeting the AKT pathway	AKT/GSK3β/Fyn/NRF2 axis	Validated in cell and animal models	Alleviated sepsis-induced acute liver injury	[146]
Patelet NLRP6 inhibition	NLRP6–TRIM21/TAB1 axis	Validated in NLRP6 knockout mice	Reduced microvascular thrombosis in sepsis	[147]
Dapagliflozin	PI3K/Akt anti-apoptotic pathway	Validated in rat and cell models	Improved sepsis-induced cardiac dysfunction	[148]
FGR kinase inhibitor	SIRT1/PGC-1α pathway	Validated in mouse models	Improved cognitive dysfunction associated with sepsis	[149]
Rhodiola rosea extract	Inhibition of the PI3K-AKT pathway	Validated in cell and animal models	Suppressed inflammation, oxidative stress, and apoptosis in sepsis	[150]
FGF1	Inhibition of the IL-6/STAT3 pathway	Validated in cell and animal models	Alleviated coagulation dysfunction and liver injury in sepsis	[83]
ELT-mediated metabolic reprogramming	Regulation of macrophage polarization	Validated in cell and animal models	Improved sepsis-induced ALI	[151]
IL-40 gene knockout	Inhibition of S100A8/9-positive neutrophils	Validated in clinical cohorts and animal models	Reduced mortality in sepsis	[152]
Xiaochaihu decoction	Inhibition of the ZBP1–PANoptosis pathway	Validated in cell and animal models	Improved sepsis-associated cardiac function and inflammation	[153]
TIFA gene knockout	Mitochondrial damage-induced pyroptosis	Validated in cell and animal models	Alleviated sepsis-induced renal injury and inflammation	[154]
Gelsevirine	Inhibition of the STING–pyroptosis pathway	Validated in animal and cell models	Improved sepsis-associated encephalopathy	[155]
MSM immunomodulatory therapy	Lactate–H3K18la–Arg1 pathway	Validated in animal and cell models	Reduced sepsis-associated systemic inflammatory response	[156]
Activation of the VDR/FFAR2 axis	Inhibition of macrophage ferroptosis	Validated through bioinformatics analysis and cell experiments	Alleviated sepsis-associated lung injury and inflammation	[157]
Bacterial ATP release blockade	Inhibition of bacterial ATP synthase and disruption of outer membrane integrity	Validated in animal and bacterial models	Attenuated neutrophil activation during sepsis	[158]
Mollugin anti-inflammatory therapy	Inhibition of TAK1–NF-κB/MAPKs and activation of Nrf2	Validated in mouse CLP sepsis model	Alleviated pulmonary and hepatic inflammation in septic mice	[79]

Note: Nrf2, nuclear factor erythroid 2-related factor 2; NLRP3, NLR family pyrin domain containing 3; IL-1β, interleukin-1 beta; EphA4, ephrin type-A receptor 4; Fc, fragment crystallizable; HO-1, heme oxygenase-1; NF-κB, nuclear factor kappa-B; UDCA, ursodeoxycholic acid; STING, stimulator of interferon genes; PANoptosis, pyroptosis-apoptosis-necroptosis; FRC, fibroblastic reticular cell; CD5L, CD5 molecule-like; PINK1, PTEN-induced putative kinase 1; CXCR1/2, C-X-C chemokine receptor type 1/2; NETosis, neutrophil extracellular trap formation; miR-223, microRNA-223; MALAT1, metastasis-associated lung adenocarcinoma transcript 1; STAT3, signal transducer and activator of transcription 3; p-STAT3, phosphorylated STAT3; Notch1, neurogenic locus notch homolog protein 1; AKT, protein kinase B; GSK3β, glycogen synthase kinase 3 beta; Fyn, Fyn proto-oncogene; NRF2, nuclear factor erythroid 2-related factor 2; NLRP6, NLR family pyrin domain containing 6; TRIM21, tripartite motif-containing protein 21; TAB1, TGF-beta-activated kinase 1 binding protein 1; PI3K, phosphoinositide 3-kinase; Fgr, Gardner-Rasheed feline sarcoma viral oncogene homolog; SIRT1, sirtuin 1; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; FGF1, fibroblast growth factor 1; IL-6, interleukin-6; ELT, esculetin; ALI, acute lung injury; IL-40, interleukin-40; S100A8/9, S100 calcium-binding protein A8/A9; ZBP1, Z-DNA binding protein 1; TIFA, TRAF-interacting protein with forkhead-associated domain; MSM, methylsulfonylmethane; H3K18la, histone H3 lysine 18 lactylation; Arg1, arginase 1; VDR, vitamin D receptor; FFAR2, free fatty acid receptor 2; ATP, adenosine triphosphate; TAK1, transforming growth factor-beta-activated kinase 1; MAPKs, mitogen-activated protein kinases; CLP, cecal ligation and puncture.

anti-inflammatory polarization of alveolar macrophages [84]. NWG specifically inhibits Src, AKT1, and cyclooxygenase-2 (COX-2) to suppress the Src/AKT1/NF-κB signaling pathway, resulting in anti-inflammatory effects and protection of lung microvascular barrier function [160]. In murine models of acute respiratory distress syndrome and sepsis, Dex@GNPs with natural glycyrrhizin protein nanoparticles can remodel the disorganized immune microenvironment and reduce tissue damage, presenting an effective method for localized anti-inflammatory and immunity regulation [161]. Dragon's blood pigment, a CMPK2 blocker, may be therapeutically effective in sepsis by regulating metabolic and signaling pathways [162]. Enoxolone-mediated sepsis suppression involving the NF-κB pathway and MEK/ERK signaling pathways suggests a new therapeutic approach that combines precise control of cellular signal transduction with anti-inflammatory treatment strategies [163]. Furthermore, miR-223 overexpression in macrophages hinders their polarization toward the M1 phenotype after LPS stimulation and decreases sepsis severity in the context of IL-4 pretreatment. These findings provide new insight into the induction of anti-inflammatory macrophages via regulation of cellular energy metabolism and provide a basis for macrophage-based cell therapy in sepsis [144].

The balance between immune activation and immunosuppression also depends on the pathogenesis of sepsis, and immunomodulation is the main therapeutic direction in infected patients. Here, recent major treatment strategies for sepsis are systematically characterized: immune checkpoint blockade, cellular and cytokine interventions, and targeted drug-delivery systems in **Figure 2**. In particular, PD-1/PD-L1 and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) inhibitors reverse immunosuppression and restore T cell effector function; thymosin α1 and cell-based products enhance host immune defense; GM-CSF and IFN-γ alleviate immune paralysis; and the modulation of macrophage M1/M2 polarization via pharmacological agents or nanoparticle delivery systems is involved in the precision treatment of the inflammatory response. Collectively, these strategies achieve targeted regulation of key nodes within the inflammatory network, suppressing excessive inflammation while preserving host defense mechanisms and multiorgan homeostasis, thereby providing novel theoretical frameworks and practical pathways for multi-level, controllable intervention in sepsis. While novel immunomodulatory therapies (melittin, GDF15, NWG, 4-OI, and nanoparticle-mediated delivery systems) show great promise in animal models of sepsis/sepsis-associated encephalopathy as well as in their in vitro counterpart, there is still a lack of clinical validation in patients, and few have been validated in national cohort studies with large sample sizes suggesting high specific efficacy. Specifically, the diagnostic value of GDF15 and its potential as a therapeutic target in sepsis-associated encephalopathy merit further experimental and clinical studies [164]. Furthermore, safety issues, dose optimization, pharmacokinetics, and patient heterogeneity require thorough evaluation in well-designed clinical trials. As such, these approaches should currently be considered promising therapeutic candidates rather than established clinical options.

6 DISCUSSION

This review comprehensively demonstrates the complex network properties of sepsis and the cytokine storm, including immune hyperactivation, cytokine networks, intercellular signaling crosstalk, microcirculatory dysfunction, metabolic remodeling, and multimodal cell death, revealing their multidirectional pathological mechanisms. As the core mechanism, the cytokine storm not only accelerates multiple organ dysfunction but also further promotes immune imbalance through a positive-feedback mechanism, thereby limiting the effectiveness of single-target interventions. Inhibition of the expression or activity of DNA-PKcs may be a promising strategy for treating sepsis, as it may inhibit or mitigate mitochondrial dysfunction and organ damage in sepsis-related MODS.

Current treatment for sepsis is mainly limited to anti-infective therapy, fluid resuscitation, and organ support. However, these strategies focus one-sidedly on the symptoms and complications of the disease, which is insufficient to regulate the complicated inflammatory process. The pathophysiology of sepsis

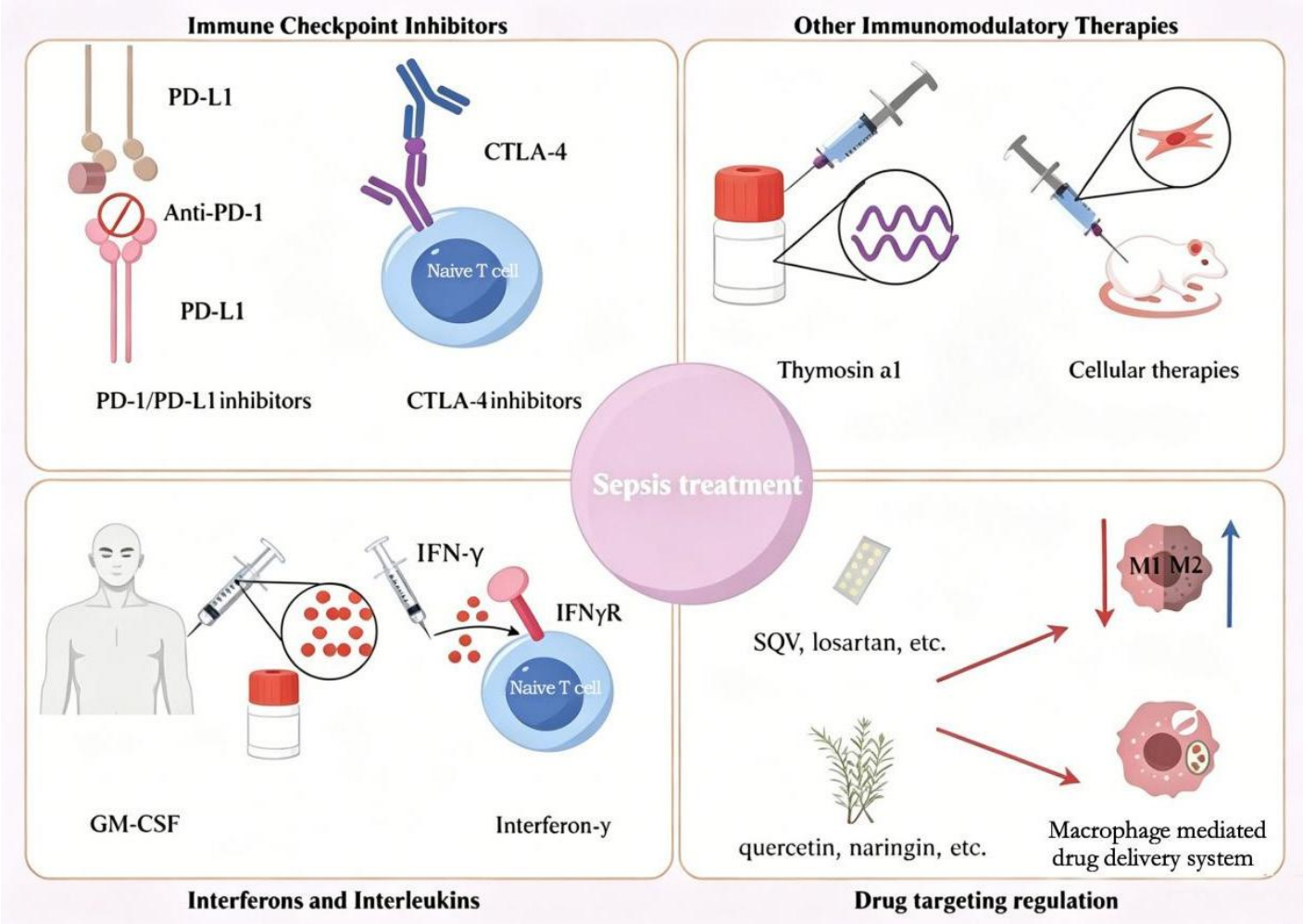

Figure 2. Immunomodulation and targeted intervention in sepsis. This figure shows the main immunomodulatory and targeted therapy strategies for treating sepsis. Anti-PD-1/PD-L1 and CTLA-4 blocking antibodies, as immune checkpoint inhibitors, can restore T cell function and ameliorate post-septic immunosuppression. Other immunomodulatory treatments, such as thymosin α1 or cellular therapy, also improve host immune defense and tissue recovery. Interferons and interleukins, such as GM-CSF and IFN-γ, promote immune cell activation and alleviate immune dysfunction. In drug-targeted regulatory strategies, the use of pharmacological agents (such as SQV, losartan, and quercetin), naringin, or macrophage-based drug delivery systems to regulate the balance between M1/M2 polarization can fine-tune the induction of inflammatory responses and favor the resolution of immune homeostasis re-establishment.

consists of signaling networks and metabolic systems interacting at multiple levels, and its nonlinear and dynamic nature suggests that single interventions may be insufficient to entirely suppress inflammatory cascades or return the immune system to homeostasis. Thus, multi-target and controllable interventions have been considered an important future direction. For instance, 4-OI has multi-target protective effects on LPS-induced septic AKI by inhibiting the inflammatory response and oxidative stress as well as promoting mitophagy [165].

Biomarkers and precision medicine hold promise in sepsis care. Early identification of high-risk patients, dynamic tracking of inflammation status, and incorporation of combined features of cytokine signatures, immune cell function, and metabolism may open options for stratified interventions and provide a support system for precise clinical decisions. The presence of different sepsis subphenotypes, as well as their relationship to disease trajectory, clinical presentations, and outcomes, has been identified through large-scale proteomics studies, leading the way to new biomarkers and precision-based interventions.

New immunomodulatory agents and anti-inflammatory interventions also offer intriguing treatment options for sepsis. For example, NAD(H)-loaded nanoparticles can enhance energy supply and inhibit inflammation, as well as promote immune homeostasis and vascular function [166]. Obeticholic acid also suppresses pro-inflammatory mediator synthesis and mitochondrial damage by reducing oxidative stress through inhibition of the NF-κB/NLRP3 signaling pathway [167]. Taken

together, these results argue for a paradigm shift in sepsis therapy, incorporating multi-level, dynamically controlled network intervention paradigms and moving away from rigid single-target approaches.

However, translational efforts and clinical practice still encounter several difficulties, such as the large heterogeneity of inflammatory networks, individual variability in immunological responses, and limitations of existing biomarkers. Notably, the combination of high-throughput omics technologies, single-cell analyses, and AI-based predictive models is anticipated to enhance early risk stratification and precision intervention, as well as to provide a theoretical basis for multi-target combination therapies [168]. Importantly, a study that utilized the Surviving Sepsis Campaign guidelines and applied machine learning to integrate six major treatments—including antibacterial therapy, balanced crystalloids, insulin therapy, corticosteroids, vasopressin, and bicarbonate—into a bundled algorithm, which significantly decreased 28-day mortality in patients with sepsis and septic shock. These findings indicate that, in the future, improving outcomes will be achieved predominantly through integrating network-based multidimensional interventions with precision medicine strategies.

ABBREVIATIONS

4-OI, 4-octyl itaconate; AKI, acute kidney injury; ALI, acute lung injury; ASC, apoptosis-associated speck-like protein containing a CARD; BMP9, bone morphogenetic protein 9; COX-2, cyclooxygenase-2; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; EV, extracellular vesicle; GM-CSF, granulocyte-macrophage colony-stimulating factor; GSDMD, gasdermin D; IDO1, indoleamine 2,3-dioxygenase 1; LPS, lipopolysaccharide; Mac-1, macrophage-1 antigen; MAPK, mitogen-activated protein kinase; MDSC, myeloid-derived suppressor cell; MODS, multiple organ dysfunction syndrome; MyD88, myeloid differentiation primary response 88; NET, neutrophil extracellular trap; NLRP3, NOD-like receptor family pyrin domain containing 3; NO, nitric oxide; NWG, N-(1,3,4-oxadiazol-2-yl) guanidine; PTX3, pentraxin 3; SOFA, sequential organ failure assessment; TIGIT, T cell immunoreceptor with Ig and ITIM domains; TLR, Toll-like receptor; Treg, regulatory T cell.

DECLARATIONS

Author contributions

Linzhu Li and Kaikai Wang contributed equally to this work and share first authorship. Linzhu Li and Kaikai Wang were responsible for literature retrieval and manuscript drafting. Yongbo Li participated in the design of the article framework and manuscript revision. Wenrong Zhang, Jianlong Ma, and Wenzhi Zhang were responsible for literature screening, summarizing research progress, and content organization. Wanquan Guo and Qianqian Zhang participated in literature analysis, figure design, and language polishing. Zhijing Song was responsible for the overall study design, academic supervision, manuscript review, and final approval of the manuscript. All authors have read and approved the final version of the manuscript.

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Perioperative Precision Medicine

Zentime

REVIEW ARTICLE

Combining traditional medicine with modern medicine: The role of acupuncture anesthesia in multidisciplinary pain management

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Abstract

Perioperative pain management remains challenging, as inadequate analgesia may delay recovery and increase opioid exposure. Multidisciplinary pain management (MPM) emphasizes multimodal and collaborative strategies, creating opportunities for integrating non-pharmacological interventions. Acupuncture anesthesia (AA), derived from traditional Chinese medicine, has re-emerged as a potential adjunct in perioperative care through modulation of nociceptive transmission, stress responses, and opioid requirements. This review summarizes the historical development, proposed neuroimmune mechanisms, and current clinical applications of AA in modern perioperative settings. Evidence from randomized controlled trials and meta-analyses suggests that AA may reduce postoperative pain scores, opioid consumption, and postoperative nausea and vomiting, and facilitate recovery in selected procedures. Moderate-level evidence supports its adjunctive role in gastrointestinal, orthopedic, and gynecological surgeries, whereas evidence in thoracic, cardiac, and highly invasive procedures remains limited and inconsistent. Critically, current evidence is constrained by small sample sizes, heterogeneous acupuncture protocols, inadequate blinding, regional concentration of studies, and insufficient long-term outcome reporting. These limitations reduce external validity and hinder guideline-level recommendations. Overall, AA shows promise as part of MPM pathways, particularly in enhanced recovery protocols, but standardized intervention protocols and large multicenter high-quality trials are needed before broader implementation in perioperative anesthesia practice.

Keywords: Acupuncture anesthesia, Perioperative pain management, Multimodal analgesia, Neuroimmune modulation, Opioid-sparing strategy

1 INTRODUCTION

Pain is one of the most common and challenging symptoms in clinical practice. Especially in the perioperative environment, insufficient pain control may adversely affect surgical results, recovery quality, and patient satisfaction. Despite advances in anesthesia technology, perioperative pain management in many patients is still not ideal, and is often affected by opioid-related adverse reactions, delayed recovery, and prolonged hospitalization [1]. In response to these challenges, the application of multidisciplinary pain management (MPM) in perioperative medicine is increasingly widespread, emphasizing coordination and cooperation between anesthesiologists, surgeons, nurses, rehabilitation specialists, and other medical professionals to optimize analgesic effects and functional recovery [2-4]. In perioperative nursing, MPM is different from its application in chronic pain management. The goal of perioperative MPM is not to focus on long-term psychosocial rehabilitation, but to achieve effective intraoperative and early postoperative analgesia, reduce perioperative stress response, minimize the use of opioids, and support enhanced recovery after surgery (ERAS) pathways [4-6]. However, the implementation of perioperative

MPM still faces major obstacles, including limited interdisciplinary training, inconsistency of clinical programs, and overreliance on drug interventions, which together limit the development of comprehensive, patient-centered analgesic strategies [3, 7].

Acupuncture anesthesia (AA) is a unique non-drug technology that has been incorporated into perioperative clinical practice since the middle of the 20th century, especially in China [1, 8, 9]. AA is rooted in the theory of traditional Chinese medicine (TCM) and is increasingly explained through modern neurophysiological frameworks. It involves the use of acupuncture stimulation as an anesthesia or anesthesia adjuvant method during surgery. It is important to note that AA should be distinguished from acupuncture for pain relief, which is mainly used for postoperative or chronic pain relief. In the perioperative context, AA aims to regulate nociceptive transmission, reduce surgical stress response, and support intraoperative and early postoperative analgesia, usually as part of a multimodal anesthesia regimen rather than as a standalone anesthesia technique [1].

More and more evidence shows that AA can affect perioperative analgesia through a variety of mechanisms, including regulating the release of endogenous opioids, regulating central and peripheral neurotransmitters, and regulating neuroimmunity and autonomic nervous system [8, 10]. Clinically, it is reported that AA can reduce the dosage of anesthetics and opioids, reduce the amount of bleeding during surgery, and promote the postoperative recovery of specific surgical populations [11, 12]. However, the clinical efficacy of AA varies between different surgeries and patient groups, and its integration in the modern perioperative path is still uneven. In addition, there is still uncertainty about standardized protocols, appropriate indications and the strength of supporting evidence, which highlights the need for systematic evaluation [11, 13].

In this review, we focus on the role of AA in perioperative pain and anesthesia management. We have summarized its historical development, the basis of the mechanism related to perioperative analgesia, its clinical application in various surgical environments, and its integration in the multidisciplinary perioperative nursing model. Furthermore, we also critically discussed the current limitations, implementation obstacles and future research directions, aiming to clarify the potential value and challenges of integrating AA into contemporary perioperative practice.

2 LITERATURE SEARCH STRATEGY AND STUDY SELECTION

A systematic literature retrieval was carried out to identify evidence related to AA and perioperative-based anesthesia and pain management interventions. Data were retrieved from PubMed, Web of Science, Embase and Wanfang from the beginning of the database establishment to December 31, 2025. Search terms were developed using controlled vocabulary (when available) and free-text keywords, including “acupuncture anesthesia” OR “acupuncture-assisted anesthesia” OR “electroacupuncture”, combined with perioperative/anesthesia-related terms such as “perioperative” OR “intraoperative” OR “anesthesia” OR “analgesia” OR “enhanced recovery”. The reference list of eligible papers and relevant reviews was manually screened to identify other studies. A representative PubMed search strategy is provided in Supplementary Table 1.

Eligible studies included clinical trials (randomized or non-randomized), observational studies, and relevant experimental studies published in English or Chinese that examined AA or acupuncture-based interventions used as anesthetic adjuncts in perioperative or intraoperative settings and reported anesthesia- or analgesia-related outcomes (e.g., pain intensity, opioid consumption, anesthetic requirements, recovery indicators, or adverse events). Studies that only focus on chronic pain management or postoperative acupuncture analgesia without clarifying the correlation of the perioperative period and the conference summary were excluded. The case report was not used as the main evidence, but it can be selectively cited when appropriate to illustrate the historical feasibility. First, the title and abstract were screened, and then the full text was evaluated for suitability. Screening and eligibility assessment were conducted by a single reviewer (C.Q.). Included studies were summarized by perioperative phase (preoperative, intraoperative, postoperative), surgical context, and study design. The quality of the study and the intensity of evidence are interpreted according to the methodological rigor (such as randomization, blindness, sample size and result report), and caution is maintained when drawing conclusions from low-level evidence. Only a small number of non-perioperative studies were cited to support the mechanism or background discussion, and they did not serve as the main evidence of perioperative clinical conclusions. This review is a narrative/qualitative synthesis based on structured retrieval, and not an informal systematic review.

3 CONCEPTUAL FRAMEWORK OF AA IN THE PERIOPERATIVE CONTEXT

3.1 Definition and scope of AA

AA refers to the use of acupuncture stimulation as anesthesia or anesthesia-assisted technique in the perioperative period, especially during and postoperative and postoperative period [14, 15]. In modern clinical practice, AA is rarely used as a separate anesthesia method; on the contrary, it is usually integrated into a multi-mode anesthesia strategy to enhance analgesia, reduce the need for drug anesthesia, and regulate the physiological stress response during the perioperative period [12, 16, 17]. In the context of the perioperative period, the main

goals of AA include reducing the transmission of injury sensations during surgery, stabilizing autonomic and neuroendocrine responses, and promoting postoperative recovery [14]. These effects are achieved through targeted stimulation of specific acupoints, usually combined with modern anesthetics, under the supervision of the perioperative team led by the anesthesiology department. Therefore, AA should be understood as an integral part of perioperative anesthesia management, not an isolated alternative therapy [17].

3.2 Distinction between AA and acupuncture analgesia

A clear conceptual distinction must be made between AA and acupuncture analgesia, because the confusion of these two methods leads to inconsistency in clinical interpretation and research design. Acupuncture analgesia is mainly used for postoperative pain control or chronic pain management, which is usually carried out outside the operating room. Its main goal is to achieve long-term pain relief, function improvement and symptom adjustment through repeated treatment [13].

In contrast, AA is especially implemented in the perioperative period, focusing on intraoperative analgesia and postoperative immediate pain regulation. The result indicators of AA emphasize the effect of anesthesia saving, intraoperative hemodynamic stability, stress response reduction and early recovery indicators, rather than long-term pain results. Failure to distinguish between the two methods may lead to improper derivation of evidence from chronic pain studies to perioperative anesthesia practice.

3.3 Role of AA in multimodal perioperative analgesia

From the perspective of modern anesthesiology, AA is most suitable as a non-drug component of multi-mode perioperative analgesia. By acting on the central and peripheral injury receptor pathways, autonomic nervous system regulation and neuroimmune interactions, AA may supplement the effect of drug anesthetics and analgesics [10, 16]. This integration is particularly relevant in strategies to reduce opioid use and ERAS programs [14, 17]. Importantly, the effectiveness of AA is affected by many factors, including the type of surgery, stimulation techniques (e.g., manual acupuncture and electric acupuncture), patient characteristics, and the time of intervention in the perioperative workflow [13, 18]. Therefore, standardized definitions and clear clinical indications are crucial to evaluate the role of AA in modern perioperative anesthesia management.

4 HISTORICAL PERSPECTIVE

4.1 The evolution of acupuncture toward modern perioperative practice

Acupuncture is one of the oldest therapeutic methods developed from Chinese medicine. Its traditional concepts of pain control and homeostasis have been documented in early medical literature such as the *Huangdi Neijing* (*The Yellow Emperor's Inner Canon*). During successive dynasties, clinical experience continuously accumulated and new techniques were developed, which laid the historical groundwork for later medical adaptation. Although these classical theories were not developed within the framework of modern anesthesiology, they still formed a conceptual basis for somatosensory modulation of pain sensation and stress responses.

In the mid-twentieth century, acupuncture entered a stage where it began to interact more intensively with biomedical science and perioperative medicine. Since the 1950s, some techniques have been introduced into clinical research and standardized practice, leading to their application in hospital settings including perioperative care [19]. Advances in physiology, neuroscience, and clinical trial methodology have allowed acupuncture-related interventions to be assessed using modern scientific tools. For example, neuroimaging methods such as functional magnetic resonance imaging have enabled us to investigate central pain processing networks, whereas device-assisted stimulations such as electroacupuncture (EA) make it possible to control stimulation intensity and frequency parameters [20-22]. This has improved reproducibility and provided a methodological basis for studying acupuncture within the context of anesthesiology.

As acupuncture has become widely used in the treatment of various diseases, perioperative medicine has increasingly focused on how to better improve analgesic effects, reduce anesthetic drug usage, and help patients recover faster before, during, and after surgery. Therefore, AA can be regarded as an extension of acupuncture into the perioperative medical field, meaning that it works together with anesthetic drugs rather than focusing solely on its therapeutic efficacy. Thus, understanding the historical background of acupuncture and moxibustion helps to understand the details of AA's emergence and development. Furthermore, knowing how modernized acupuncture has developed toward standardization and mechanistic understanding can also help us realize why perioperative pain control still focuses so much on AA today. The historical evolution and major milestones of AA in perioperative medicine are summarized in **Figure 1**.

4.2 The application of acupuncture in integrative and perioperative medicine

Acupuncture is one of the most widely used forms of TCM. Acupuncture can stimulate specific acupoints and regulate body function to achieve the purpose of preventing and treating diseases. With the development of modern medical science, especially neurophysiology and immunology, the mechanism of acupuncture has been further studied. Studies have shown that acupuncture acts on peripheral and central nerve pathways. Acupuncture can also regulate the release of neurotrans-

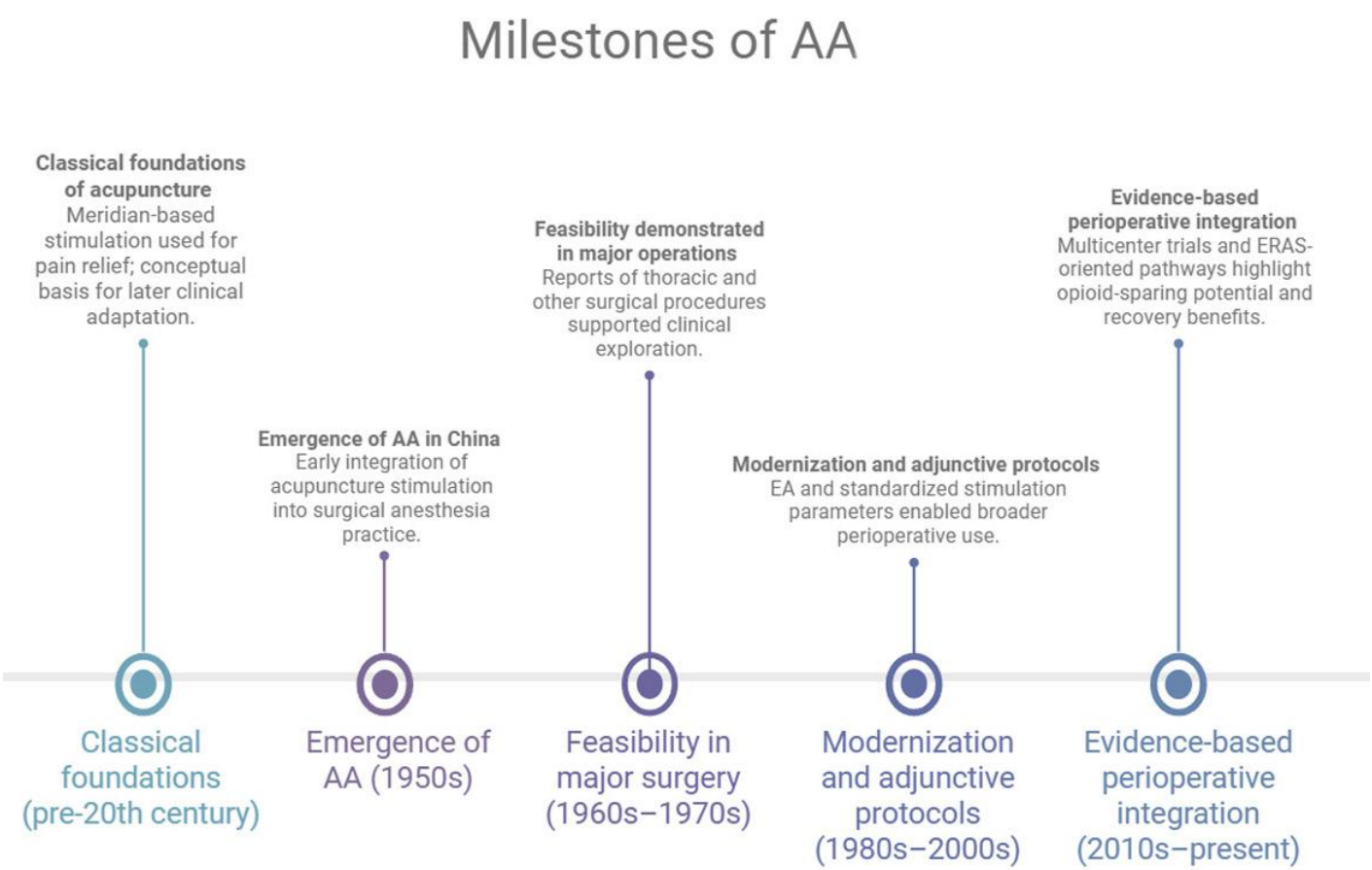

Figure 1. History and key milestones of acupuncture anesthesia in perioperative medicine. The timeline highlights the conceptual basis of acupuncture in pain regulation, the emergence of AA in the 1950s, early feasibility reports in major surgeries, and subsequent modernization through electroacupuncture protocols and parameter standardization. In recent years, AA has been increasingly evaluated and incorporated as an adjunctive method for multimodal perioperative analgesia and ERAS-oriented treatment pathways, focusing on reduced opioid use and recovery-related outcomes. Created by the author using BioRender. AA, acupuncture anesthesia; EA, electroacupuncture; ERAS, enhanced recovery after surgery.

mitters, inflammatory factors, and other mediators. It can also affect the patient's stress response and reduce pain and other rehabilitation-related processes [19, 23]. Therefore, acupuncture-based interventions can be better integrated into modern medicine.

From the perspective of perioperative medicine, its clinical focus differs from that of general outpatient indications: perioperative application prioritizes analgesia, regulation of surgical stress response, and reduction of the drug burden in the anesthesia pathway. In this context, AA represents a structured perioperative-oriented application that can be implemented before, during, or after surgery as part of a multimodal analgesia strategy. Technological advances—particularly EA—have standardized the control of stimulation frequency and intensity, improved repeatability, and promoted integration with the perioperative workflow. In addition, ear-based methods and acupoint stimulation protocols have been explored as adjunctive strategies for the management of perioperative symptoms, including postoperative pain and nausea, although standardization is still a key requirement for wider implementation.

In Western medical practice, the adoption of acupuncture is closely linked to evidence-based evaluation and mechanistic research. Neuroimaging, biochemical assays, and perioperative outcome studies have been used to test the regulatory effects of acupuncture on the nervous, endocrine, and immune systems [23, 24]. This type of mechanistic research provides a stricter biological basis for clinical use and supports the development of standardized perioperative protocols. In addition, the use of devices and minimally invasive methods (such as EA and related stimulation techniques) offer practical advantages in the perioperative environment, as they allow parameter setting and improve consistency of operation. Overall, the growing evidence base and increasing methodological rigor help to position acupuncture-based interventions, including AA, as potential adjunctive approaches for modern perioperative pain management and holistic anesthesia care.

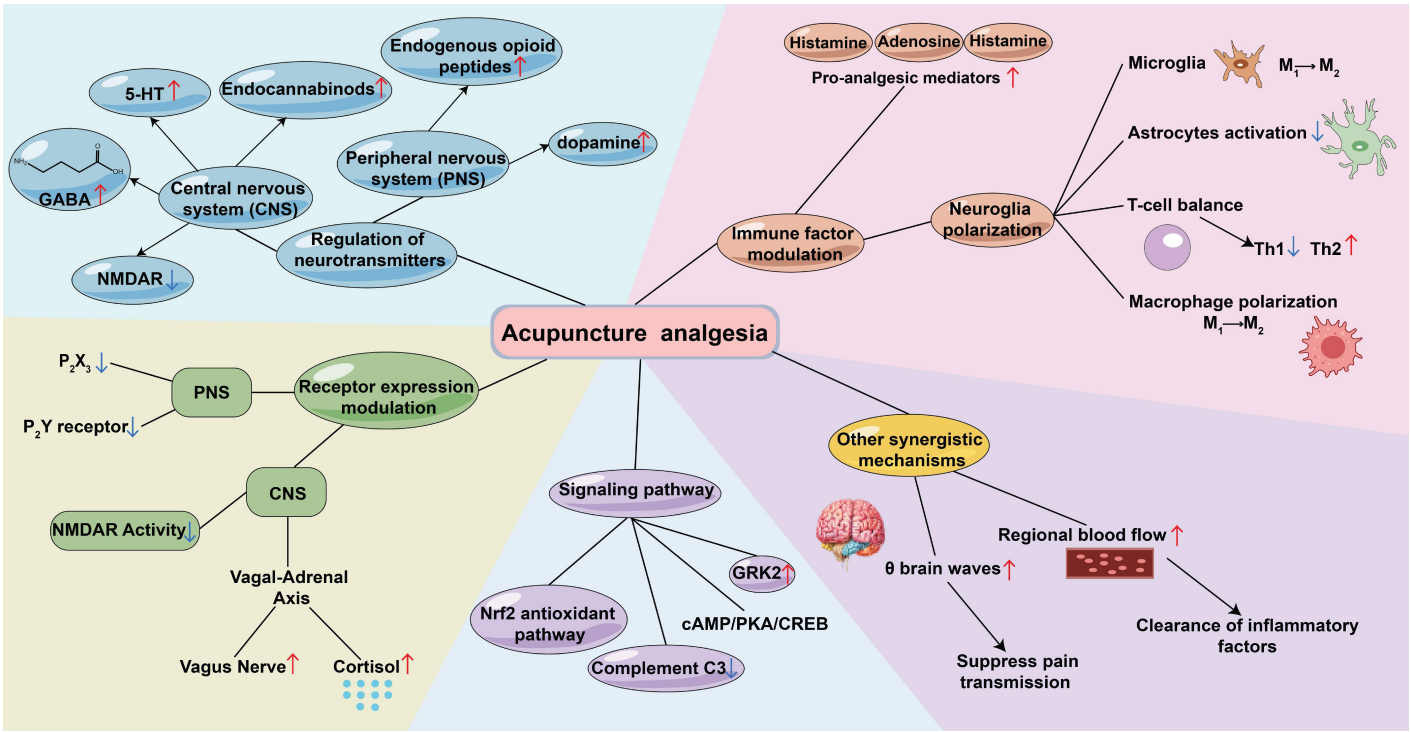

Figure 2. Schematic overview of mechanisms underlying acupuncture-mediated analgesia relevant to perioperative care. Acupuncture stimulation modulates interconnected central and peripheral pathways, including neurotransmitter regulation, receptor activity (e.g., purinergic receptors and NMDAR), immune and neuroendocrine responses, and intracellular signaling cascades. Additional systemic effects, such as vagal–adrenal activation and enhanced clearance of inflammatory mediators, may further contribute to reduced nociceptive signaling and perioperative pain. Created by the authors using BioRender. 5-HT, 5-hydroxytryptamine; GABA, γ-aminobutyric acid; NMDAR, N-methyl-D-aspartate receptor; PNS, peripheral nervous system; CNS, central nervous system; P_2X_3/P_2Y, purinergic receptors; Nrf2, nuclear factor erythroid 2-related factor 2; GRK2, G protein-coupled receptor kinase 2; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; CREB, cAMP response element-binding protein; Th1/Th2, T helper 1/2 cells.

4.3 Applications of AA in modern perioperative medicine

Acupuncture has long been used for pain regulation. Contemporary practice has expanded from manual body acupuncture to include EA, ear acupuncture stimulation, and related acupoint stimulation techniques [19, 25, 26]. These technological advances have particular relevance to perioperative care. For perioperative care, reproducibility, parameter control, and integration into the entire anesthesia process are all very important aspects. In this context, AA is now regarded more as an additional tool than as an independent anesthesia modality, supporting multimodal analgesia strategies and reducing opioid use.

Evidence from both clinical and physiological studies has shown that acupuncture can modify pain processing and the response to surgical stress during the perioperative period. It does so through its regulation of neural transmission pathways and immune system pathways involved in nociception and inflammation. Acupuncture has been applied in surgical settings and studied for the following outcomes: reducing postoperative pain severity; decreasing opioid requirement; and lessening anesthesia-related side effects, including postoperative nausea and vomiting (PONV) [19, 27]. It should be noted that perioperative use of acupuncture requires a clear distinction between intraoperative and postoperative applications, as these two approaches differ in timing, objectives, and coordination with general anesthesia management.

Although clinical interest has increased, the widespread use of AA in conventional medicine still faces technical and efficacy-related obstacles. The diversity of acupoint selection, stimulation parameters (frequency/intensity), timing of intervention, and outcome reporting reduces study comparability and makes evidence synthesis difficult. Therefore, standardized AA protocols and high-quality trial results are still needed to establish indications, determine optimal procedures, and integrate AA into ERAS pathways. Beyond clinical evaluation, improvements in objective monitoring and quantitative methods—such as standardized outcome reporting and stable stimulation protocols—will help build stronger evidence for the scientific implementation of acupuncture in perioperative pain management.

In brief, acupuncture has evolved from classical theoretical foundations to relatively standardized and scientific research. Currently, increasing attention is being paid to its perioperative applications, particularly its role in regulating pain perception, reducing surgical stress response, decreasing opioid consumption, and supporting postoperative recovery. Nevertheless, modern evidence-based medicine still faces challenges in standardization, reproducibility, and mechanistic clarification. As more acupuncture-related interventions are increasingly integrated into perioperative multidisciplinary care systems, a better understanding of their neurophysiological and molecular mechanisms will help develop more scientific protocols and facilitate clinical translation. Therefore, the following section summarizes the existing evidence on the mechanisms and pathways of acupuncture analgesia and perioperative regulation.

5 MECHANISMS OF ACTION

Acupuncture stimulation produces perioperative analgesia and anesthesia auxiliary effects by activating specific acupoints. Its mechanism of action is multifaceted, involving the coordinated regulation of the peripheral nervous system and the central nervous system (CNS), as well as the interaction with the immune, endocrine and stress response pathways [8]. In the context of the perioperative period, these mechanisms jointly help to weaken the sensory conduction of injury, stabilize the physiological response to surgical stress, and enhance the effect of anesthesia.

As summarized in **Figure 2**, acupuncture-induced analgesia produces analgesic effects by regulating neurotransmitter release, modulating immune and inflammatory mediators, altering receptor expression, and activating specific intracellular signaling pathways [28-30]. Rather than acting through a single pathway, acupuncture-induced analgesia influences multiple interconnected systems that are highly relevant to perioperative pain processing and anesthesia management.

5.1 Regulation of neurotransmitters

As a distinct non-pharmacological component of perioperative analgesia, acupuncture modulates neurotransmitter activity in both the central and peripheral nervous systems, thereby influencing pain perception and stress-related responses during surgery.

As illustrated in **Figure 3**, needling at commonly used acupoints such as Large Intestine 4 has been shown to activate γ-aminobutyric acid (GABA) receptors in the CNS. GABA is the primary inhibitory neurotransmitter in the CNS and plays a critical role in suppressing excessive neuronal excitability. Acupuncture-induced activation of GABAergic signaling reduces neuronal excitability in the spinal dorsal horn, a key relay center for nociceptive transmission, thereby attenuating ascending pain signals during the perioperative period [28]. Acupuncture has also been reported to influence serotonergic signaling by activating 5-hydroxytryptamine (5-HT) receptors and promoting 5-HT release [31-33]. Serotonin participates in descending inhibitory pain pathways and contributes to the modulation of pain perception and affective responses. Increased central 5-HT availability may suppress nociceptive transmission and alleviate perioperative anxiety and stress, which are known to amplify pain perception [28, 34, 35]. It should be noted, however, that variations in surgical models, stimulation parameters, and timing of intervention may account for inconsistent findings regarding serotonergic changes across studies, highlighting the need for standardized perioperative protocols.

In addition, acupuncture modulates the endocannabinoid system and N-methyl-D-aspartate receptor (NMDAR) signaling pathways, particularly within supraspinal regions involved in pain modulation, such as the anterior cingulate cortex (ACC) [36, 37]. Although ACC hyperactivity is more extensively characterized in chronic pain states, perioperative noxious stimulation can also engage affective–cognitive pain networks. Acupuncture has been shown to increase levels of endocannabinoid ligands, including anandamide and 2-arachidonoylglycerol, as well as cannabinoid type 1 receptor expression, thereby reducing neuronal hyperexcitability and contributing to analgesic effects [28, 36]. Regulation of NMDAR signaling further limits excessive excitatory transmission and may help prevent perioperative central sensitization [36, 38].

At the peripheral level, acupuncture induces the release of endogenous opioids such as β-endorphin and enkephalin [32, 39]. These endogenous peptides bind to opioid receptors and inhibit nociceptive signal transmission in a manner analogous to exogenous opioids, but without the risks of tolerance and dependence [8, 28, 40]. This mechanism provides a biological basis for the opioid-sparing effects observed in perioperative settings [38]. Moreover, acupuncture has been shown to modulate dopaminergic activity [31, 41]. Dopamine plays an important role in reward processing, motor control, and emotional regulation, and its modulation may indirectly influence pain perception by alleviating negative affective states associated with surgical stress and pain [34, 42].

5.2 Regulation of immune and inflammatory factors

Acupuncture stimulation modulates immune and inflammatory responses that are closely associated with perioperative pain and surgical stress. Local acupuncture stimulation promotes the release of immune mediators, including histamine, adenosine, and β-endorphin, which exert analgesic effects by directly or indirectly binding to receptors on peripheral nerve endings [40]. Among these mediators, adenosine—an endogenous purine nucleoside—has been shown to significantly attenuate mechanical and thermal hyperalgesia through activation of

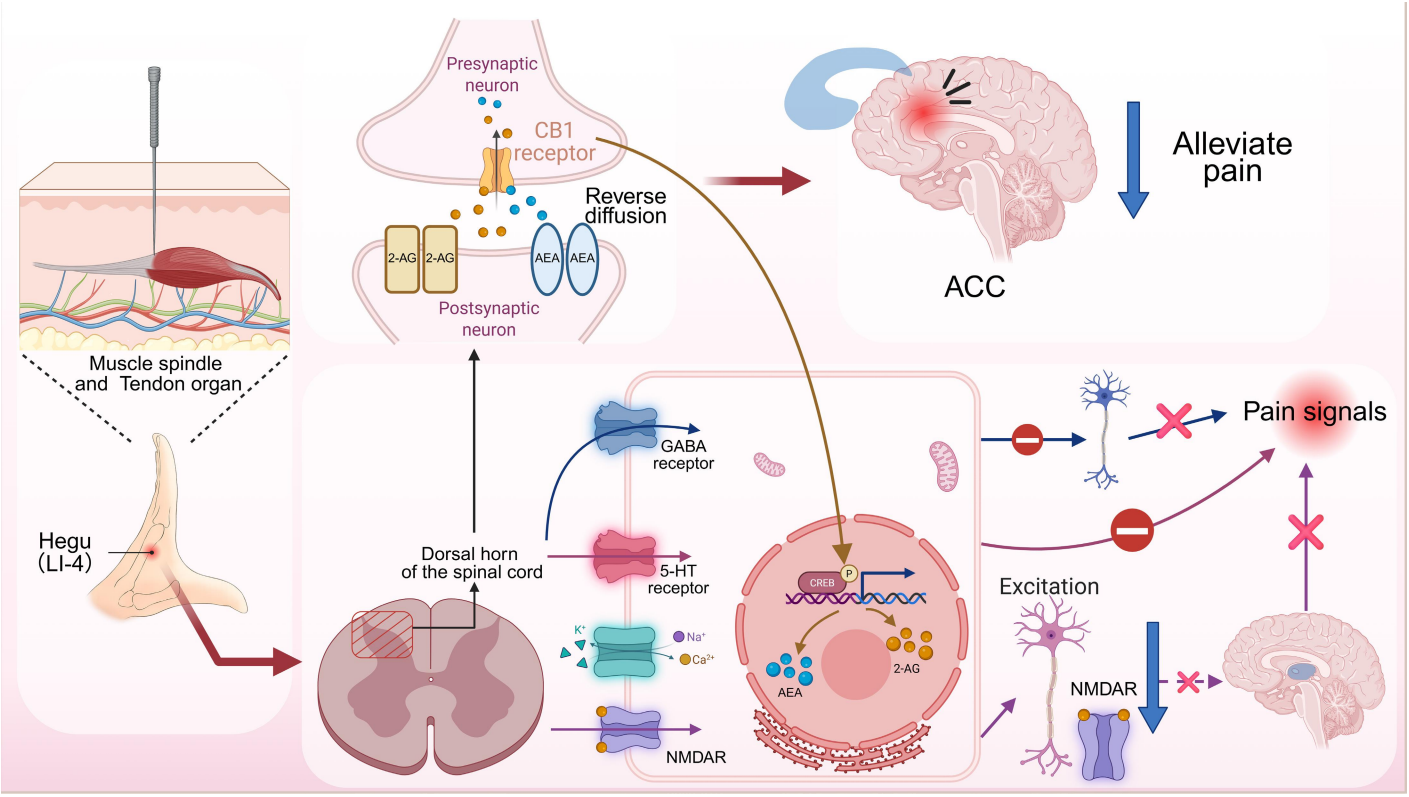

Figure 3. Multilevel central neuroregulatory mechanisms relevant to acupuncture anesthesia (electroacupuncture example). Electroacupuncture stimulation at the Hegu (LI-4) acupoint modulates pain processing at spinal and supraspinal levels. It has been reported to enhance endocannabinoid signaling and CB1 receptor activation, reducing presynaptic neurotransmitter release via retrograde signaling. In parallel, inhibitory transmission mediated by GABA and 5-HT receptors is strengthened, whereas excitatory NMDAR signaling is suppressed. Together, these effects attenuate nociceptive transmission in the spinal dorsal horn and ACC, supporting perioperative analgesia. Created by the authors using Adobe Illustrator. ACC, anterior cingulate cortex; CB1 receptor, cannabinoid receptor type 1; 2-AG, 2-arachidonoylglycerol; AEA, arachidonoylethanolamide (anandamide); GABA, γ-aminobutyric acid; 5-HT, 5-hydroxytryptamine; NMDAR, N-methyl-D-aspartate receptor; CREB, cAMP response element-binding protein; LI-4, Large Intestine 4.

adenosine receptors (A1, A2A, A2B, and A3) [40]. Although this mechanism has been extensively studied in both acute and chronic pain models, it is also highly relevant to perioperative nociception, in which tissue injury and inflammation play a central role.

At the central level, acupuncture influences neuroimmune interactions by modulating microglial activation and polarization. Microglia, the resident immune cells of the CNS, exhibit distinct functional phenotypes, including the pro-inflammatory M1 phenotype and the anti-inflammatory M2 phenotype. Acupuncture has been shown to promote a shift from the M1 to the M2 microglial state, thereby suppressing neuroinflammatory responses [29, 30, 37]. M1 microglia release pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6, which amplify neuroinflammation and enhance nociceptive sensitization. In contrast, M2 microglia secrete anti-inflammatory mediators, including interleukin-10 and transforming growth factor-β, which facilitate inflammation resolution and tissue repair [29, 39]. Through regulation of microglial polarization, acupuncture stimulation attenuates neuroinflammation and reduces central sensitization relevant to perioperative pain processing.

Acupuncture stimulation also modulates peripheral immune responses that contribute to pain and inflammation following surgical injury. T lymphocyte subsets, including T helper 1 (Th1) and T helper 2 (Th2) cells, play opposing roles in immune regulation. Th1 cells predominantly secrete pro-inflammatory cytokines such as interferon-γ and interleukin-2, whereas Th2 cells produce anti-inflammatory mediators including interleukin-4, interleukin-5, and interleukin-13. Acupuncture has been reported to rebalance the Th1/Th2 axis by reducing pro-inflammatory cytokine release and enhancing anti-inflammatory signaling [31]. In parallel, acupuncture promotes macrophage polarization from the pro-inflammatory M1 phenotype toward the anti-inflammatory M2 phenotype, further limiting inflammatory mediator production and supporting pain relief [31].

Within the spinal cord, acupuncture suppresses excessive activation of glial cells, including microglia and astrocytes, which are key contributors to neuroinflammation and pain sensitization [28]. Overactivation of these glial cells leads to increased release of inflammatory mediators such as TNF-α and IL-1β, thereby amplifying nociceptive signaling. By inhibiting glial activation and inflammatory factor release, acupuncture stimulation mitigates neuroinflammatory processes and contributes to reduced pain sensitivity during the perioperative period [29].

5.3 Regulation of receptor expression and neuroregulatory pathways

In the peripheral nervous system, acupuncture modulates the expression and activity of purinergic receptors, which play a critical role in inflammatory and nociceptive signaling. Purinergic receptors are broadly classified into ionotropic P2X receptors and G-protein-coupled P2Y receptors. These receptors are activated by extracellular adenosine triphosphate, which is released in large quantities following tissue injury and surgical trauma. Activation of purinergic receptors, including P2X3, P2X4, P2X7, and P2Y subtypes, contributes to peripheral sensitization and inflammation-associated pain [43]. Experimental evidence indicates that acupuncture downregulates the expression of purinergic receptors, thereby attenuating adenosine triphosphate-mediated activation of sensory neurons and immune cells and reducing inflammatory and nociceptive signaling [37, 44]. In particular, P2X3 receptors, which are predominantly expressed on primary sensory neurons, have been implicated in the development and maintenance of pain sensitization. Suppression of P2X3 receptor expression by acupuncture may therefore diminish peripheral nociceptive transmission and contribute to analgesia in the perioperative setting [28, 33, 44].

Within the CNS, acupuncture modulates NMDAR activity, a key mechanism involved in excitatory synaptic transmission and pain signal amplification. NMDARs are widely distributed in pain-processing regions, including the spinal dorsal horn and supraspinal structures such as the ACC. Excessive activation of NMDARs enhances excitatory neurotransmission and facilitates central sensitization. Although central sensitization is more extensively characterized in chronic pain, perioperative noxious stimulation can transiently engage similar excitatory mechanisms. By regulating NMDAR activation, acupuncture attenuates nociceptive signal transmission in the spinal dorsal horn and reduces excessive excitability in supraspinal pain-modulating regions, thereby contributing to perioperative analgesia [36, 37, 45]. The ACC plays an important role in the affective and cognitive dimensions of pain perception. Overactivation of this region is associated with heightened pain-related distress and negative emotional responses. Modulation of NMDAR signaling by acupuncture may alleviate excessive ACC excitability, thereby attenuating pain-related emotional responses during the perioperative period [34, 36-38].

In addition to receptor-level modulation, acupuncture exerts systemic anti-inflammatory and analgesic effects through activation of the vagus nerve–adrenal axis. Vagal stimulation influences autonomic balance and promotes adrenal release of anti-inflammatory hormones, including cortisol [30, 31, 39, 41]. Cortisol, a key glucocorticoid hormone, suppresses the production and release of pro-inflammatory cytokines and modulates immune responses, thereby limiting inflammation-induced pain [31]. Through this neuroendocrine pathway, acupuncture achieves coordinated peripheral and central regulation extending beyond local stimulation sites. Collectively, modulation of purinergic receptors, glutamatergic signaling, and autonomic–endocrine pathways forms an integrated biological network that contributes to perioperative analgesia.

5.4 Modulation of signaling pathway activation

Acupuncture exerts analgesic and anti-inflammatory effects through modulation of multiple intracellular signaling pathways that regulate oxidative stress, synaptic plasticity, immune responses, and neuroinflammation. In perioperative conditions, surgical trauma and ischemia–reperfusion processes are often accompanied by excessive production of reactive oxygen species, which contribute to inflammation and nociceptive sensitization. One key pathway involved is the nuclear factor erythroid 2–related factor 2 (Nrf2) signaling pathway. Under oxidative stress, Nrf2 dissociates from its cytoplasmic inhibitor Kelch-like ECH-associated protein 1, translocates into the nucleus, and binds to antioxidant response elements, thereby up-regulating transcription of antioxidant and cytoprotective genes. Acupuncture has been shown to activate the Kelch-like ECH-associated protein 1–Nrf2–antioxidant response element pathway, thus enhancing antioxidant capacity, reducing reactive oxygen species accumulation, and decreasing inflammation-related pain [34, 36, 40]. This mechanism is particularly relevant in the perioperative environment, as oxidative stress plays a key role in tissue damage and postoperative pain.

In parallel, acupuncture modulates signaling pathways involved in synaptic plasticity. The cyclic adenosine monophosphate (cAMP)/protein kinase A/cAMP response element-binding protein cascade is a central regulator of activity-dependent gene transcription and maladaptive synaptic plasticity. Overactivation of this pathway has been considered to be related to pain amplification and sensitization. By down-regulating cAMP/protein kinase A/cAMP response element-binding protein signaling, acupuncture reduces aberrant neuronal excitability and limits nociceptive signal amplification [34, 36, 38].

Acupuncture also influences neuroimmune signaling pathways associated with glial activation. Down-regulation of complement component C3 has been shown to suppress spinal micro-

Table 1. Integrated mechanistic pathways of acupuncture-mediated analgesia relevant to perioperative care

Level	Key targets	Main effects	Perioperative relevance
Neurotransmitters	GABA, 5-HT, endocannabinoids, opioids	Inhibit nociceptive transmission; reduce central excitability	↓ intraoperative anesthetic and opioid requirements; ↓ postoperative pain
Immune modulation	Microglia (M1→M2), Th1/Th2 balance	Suppress neuroinflammation; reduce cytokine release	↓ postoperative inflammatory pain
Receptor regulation	P2X3/P2Y receptors, NMDAR, CB1	Attenuate ATP-mediated nociception; inhibit central sensitization	↓ acute postoperative pain; mitigate risk of PPSP
Signaling pathways	Nrf2–Keap1–ARE, cAMP/PKA/CREB, GRK2	Antioxidant effects; regulate neuroplasticity	Attenuate perioperative stress responses; support recovery-related outcomes
Autonomic & endocrine	Vagus–adrenal axis, cortisol	Systemic anti-inflammatory effects	Stabilize perioperative homeostasis

Note: GABA, γ-aminobutyric acid; 5-HT, 5-hydroxytryptamine; Th1/Th2, T helper 1/T helper 2 cells; P2X3, purinergic P2X3 receptor; P2Y, purinergic P2Y receptor; NMDAR, N-methyl-D-aspartate receptor; CB1, cannabinoid receptor type 1; ATP, adenosine triphosphate; PPSP, persistent postsurgical pain; Nrf2, nuclear factor erythroid 2-related factor 2; Keap1, Kelch-like ECH-associated protein 1; ARE, antioxidant response element; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; CREB, cAMP response element-binding protein; GRK2, G protein-coupled receptor kinase 2.

gliosis and astrogliosis, thereby decreasing the release of pro-inflammatory mediators and mitigating neuroinflammatory responses [29, 35, 45]. Furthermore, acupuncture modulates G protein-coupled receptor kinase 2 (GRK2) expression in spinal neurons. GRK2 plays a critical role in the regulation of inflammatory signaling, and its reduced expression is associated with pain chronification. Acupuncture-induced up-regulation of GRK2 suppresses microglial activation, decreases pro-inflammatory cytokines such as TNF-α and IL-1β, and enhances anti-inflammatory cytokines including interleukin-10 and interleukin-4, collectively contributing to attenuation of inflammatory pain [28-30, 37].

Taken together, modulation of antioxidant pathways, synaptic plasticity-related signaling, and neuroimmune cascades allows acupuncture to exert coordinated analgesic and anti-inflammatory effects that are relevant to perioperative pain control.

5.5 Additional neurophysiological and systemic mechanisms

In addition to the mechanisms described above, acupuncture may engage broader neurophysiological and systemic regulatory processes. Modulation of immune–endothelial interactions and enhancement of local microcirculation have been reported to facilitate clearance of inflammatory mediators and support tissue recovery following surgical injury [29, 35-37].

At the central level, acupuncture may influence nociceptive processing by modulating cerebral oscillatory activity, including theta rhythm regulation, which has been implicated in pain perception and cognitive–affective processing of noxious stimuli [43]. This central regulation indicates that in addition to receptor- and pathway-level mechanisms, additional levels of neural regulation exist.

Psychological and emotional factors may also affect perioperative outcomes. Preoperative anxiety associated with surgery or unfamiliar anesthesia techniques may exacerbate pain perception. Acupuncture has been reported to exert a sedative effect by regulating the neurotransmitter system [41, 42]. In addition, effective communication and comfort provided by experienced clinicians may further reduce anxiety and enhance patient cooperation. Although these psychosocial factors are not the primary analgesic biological mechanisms, they may contribute to the overall perioperative experience and perception of therapeutic effect.

Collectively, these multi-level regulatory processes indicate that acupuncture modulates neural, immune, endocrine, and central integrative systems in a coordinated manner, influencing both nociceptive processing and perioperative stress responses. This integrated biological framework supports the mechanistic plausibility of acupuncture in perioperative contexts. The key molecular, neuroimmune, and neuroregulatory mechanisms discussed above are summarized in **Table 1**.

6 PERIOPERATIVE CLINICAL APPLICATIONS OF AA

Based on these mechanistic understanding, AA has gradually become part of routine perioperative pain management as a multimodal analgesia and opioid-sparing strategy [46]. Its clinical use also represents a translational extension of the neuroimmunomodulatory and regulatory mechanisms described above [46]. Originating in China more than five decades ago, AA is a non-drug anesthetic technique that integrates ancient acupuncture methods with contemporary clinical practice [47, 48]. Based on the concept of perioperative precision medicine, AA can provide individualized pain management, reduce the amount of medication used, and improve postoperative recovery efficiency. Representative clinical applications of AA in

Table 2. Perioperative clinical applications of acupuncture anesthesia and precision considerations

Surgical context	Intervention type	Clinical outcome	Precision consideration
Colorectal surgery	AA delivered via intraoperative EA	Reduced postoperative pain scores and accelerated gastrointestinal recovery	Frequency-specific stimulation and individualized acupoint selection
Orthopedic surgery (knee replacement)	AA as an adjunct to conventional anesthesia/analgesia	Reduced postoperative analgesic requirement and improved recovery profile	Tailored perioperative analgesic protocols
Thoracic surgery	AA-assisted anesthesia (selected cases)	Feasibility of maintaining intraoperative consciousness with stable physiological parameters	Multidisciplinary intraoperative monitoring
Abdominal surgery	AA delivered via EA as an adjunct to general anesthesia	Reduced complication rates and improved postoperative recovery	Optimized stimulation parameters according to patient condition

Note: AA, acupuncture anesthesia; EA, electroacupuncture.

perioperative medicine, together with precision-oriented considerations, are summarized in **Table 2**.

6.1 High-level evidence (randomized controlled trials [RCTs] and meta-analyses)

Some RCTs and meta-analyses have found that AA, mainly EA, offers multiple advantages for perioperative analgesia. These include lower postoperative pain scores, reduced opioid consumption, and fewer cases of PONV [47, 48].

Regarding colorectal surgery, previous clinical studies have suggested that perioperative EA may improve postoperative pain management and facilitate gastrointestinal recovery compared with conventional perioperative care. Some studies have reported earlier recovery of bowel function, including reduced time to first flatus and bowel movement, together with enhanced postoperative recovery outcomes. These findings support the potential role of EA as an adjunctive strategy within multimodal perioperative analgesia and ERAS protocols, although further high-quality studies are still needed to strengthen the evidence base.

Similarly, in orthopedic procedures such as knee arthroplasty, AA has demonstrated opioid-sparing effects and improved postoperative recovery, supporting its role as an effective adjunct within multimodal analgesia strategies [47-49].

6.2 Moderate-level evidence (controlled clinical and observational studies)

Moderate level evidence from controlled clinical trials and observation studies indicates that AA may provide additional benefits for a wider range of perioperative situations.

During the preoperative stage, studies have shown that AA can reduce anxiety levels, regulate stress responses, and increase patients' tolerance to surgical procedures [47, 48]. Preoperative anxiety is known to affect postoperative pain intensity and analgesic requirements [47]. From a mechanistic perspective, needling at specific acupoints can regulate neurotransmitter release and sympathetic nerve activity, thereby reducing mental stress. In clinical practice, this effect helps reduce perioperative analgesic medication use and enhance patient satisfaction [47, 48].

In the postoperative period, AA has been shown to reduce postoperative pain intensity, decrease opioid consumption, and lower the incidence of anesthesia-related side effects, including nausea and vomiting [46, 50]. For orthopedic and abdominal surgeries, acupuncture-assisted anesthesia can also enhance functional recovery and improve patient satisfaction. However, variations in study designs, intervention protocols, and outcome measures may lead to inconsistent findings across studies. Therefore, readers exercise caution when interpreting these results [46, 47].

6.3 Postoperative analgesia and recovery promotion

Evidence supporting the use of AA in highly invasive procedures, such as thoracic surgery, remains limited and largely exploratory. Clinical reports of procedures such as lobectomy performed under AA suggest that patients can maintain intraoperative consciousness with adequate analgesia and achieve satisfactory postoperative recovery. However, these findings are primarily derived from case reports or small-scale studies, which limits their generalizability [47, 48].

In addition, mechanistic evidence supporting perioperative applications of AA is largely extrapolated from studies on chronic or neuropathic pain. For example, EA has been shown to alleviate neuropathic pain through modulation of the transient receptor potential vanilloid 1 channel signaling pathway and suppression of neuroinflammation [50, 51]. While these mechanisms provide a plausible biological basis for postoperative analgesia, direct evidence in acute perioperative pain settings remains insufficient. Therefore, further high-quality studies are needed to validate these effects in clinical practice.

6.4 Multidisciplinary collaboration and integration into ERAS pathways

The perioperative use of AA requires good cooperation among all members of the team [46]. Surgeons, anesthesiologists, acu-

puncturists, nurses, rehabilitation therapists, and psychological support teams each have their own role in ensuring patient safety and comfort [46]. Preoperative multidisciplinary meetings help physicians develop individualized treatment plans for patients; intraoperative communication allows adjustment of anesthesia and surgical strategies according to patient response; and postoperative ward visitation and coordination of rehabilitation support functional recovery.

AA is one of the most promising adjunctive strategies for ERAS. Its implementation at different stages (preoperative, intraoperative, and postoperative) of the ERAS pathway is consistent with the ERAS concept of reducing surgical stress responses, minimizing opioid consumption, accelerating recovery, and improving care quality [47]. Based on standardized protocols and structured teamwork, AA can contribute to the optimization of perioperative pain management and improved patient outcomes.

7 BENEFITS OF INTEGRATING AA

7.1 Enhancing patient prognosis

The use of AA has been shown to be associated with accelerating postoperative recovery and improving perioperative results in various surgical environments. During the operation, AA can reduce the need for conventional anesthetics, which helps to improve physiological stability and promote the early recovery of consciousness and gastrointestinal function. In neurosurgical surgeries such as craniotomy, it has been reported that AA can shorten the intubation time and postoperative recovery time, while reducing the incidence of complications. AA also significantly decreases PONV, opioid consumption, and opioid-related adverse effects, thereby facilitating enhanced recovery pathways [19, 52]. By regulating the autonomic nervous system, it can reduce surgical stress, reduce the incidence of complications, accelerate recovery, shorten hospitalization time, and reduce costs [53].

In general, these perioperative benefits show that AA may improve the prognosis of patients by supporting faster recovery, reducing the drug burden and promoting physiological homeostasis during surgery [54].

7.2 Reducing dependence on medications

As a non-pharmacological intervention, AA avoids many adverse effects and dependency risks associated with drug-based analgesia. By activating the endogenous analgesic pathway, AA can reduce the dose of conventional anesthetics while maintaining sufficient perioperative analgesia. In neurosurgery such as craniotomy, research shows that AA can reduce the need for inhaled anesthetics, thus reducing the risk associated with anesthesia [52, 54]. In postoperative pain management, AA helps to reduce the use of opioids and reduce the incidence of opioid-related adverse reactions. Unlike drug analgesics, AA does not cause drug tolerance or organ-specific toxicity involving the liver, kidney or gastrointestinal system, which makes it especially suitable for patients with limited tolerance to opioids [52, 53, 55]. Compared with standard drug treatment, AA shows good safety characteristics, with fewer and milder side effects.

Additionally, AA represents a relatively simple and cost-effective intervention that does not require specialized equipment or a large amount of resources. By reducing the drug burden while maintaining analgesic effects, AA may provide a safer and more cost-effective method of perioperative pain management.

7.3 Improving patient compliance and satisfaction

AA may improve patient compliance and satisfaction through both physiological and psychosocial mechanisms. By providing analgesia while maintaining patient consciousness and physiological stability, AA encourages active patient participation and engagement during the perioperative process [53, 56]. Positive treatment expectations and reduced anxiety associated with acupuncture-based interventions may further enhance analgesic outcomes and improve perioperative comfort [19, 54, 57]. AA demonstrates a favorable safety profile, with no serious adverse events reported in most clinical studies [58, 59]. Reported side effects are generally mild and self-limiting, including minor subcutaneous bleeding or transient post-acupuncture sensations [60-62]. This safety profile contributes to increased patient confidence and adherence to perioperative treatment plans [52, 61].

Moreover, AA could increase treatment uptake among those patients who prefer complementary therapy or a non-drug treatment regimen for pain relief. Combined with conventional treatment methods, AA provides another kind of choice matching individual needs, at the same time guaranteeing a good safety and efficacy level [53, 56]. Altogether, such aspects would benefit the perioperative process, raise patient satisfaction and promote better treatment compliance.

8 CHALLENGES AND BARRIERS

There are still several problems limiting the wide application of AA as a complementary option for perioperative pain management. First, there are many types of heterogeneity among the published studies. These studies differ mainly in the surgical population, acupuncture methods, stimulation parameters, and outcome measurement. Second, although most of the RCTs have shown some positive effects of AA on perioperative outcomes, many of them suffer from small sample size, single-center design, and varying methodological quality, all of which weaken the reliability of current evidence and limit the development of guidelines. Third, because important perioperative

outcomes such as postoperative pain intensity, opioid use, and recovery quality are not consistently measured across studies, cross-study comparison and meta-analysis cannot be performed.

In terms of clinical application, AA alone can hardly meet the anesthetic requirement of all types of surgery. In cases of more invasive procedures or prolonged surgeries with greater surgical stress, AA should be applied together with other conventional anesthesia techniques, because of its poor pain-relieving effects when used alone [63, 64]. Individual differences may affect the impact brought by AA as well. Such differences could involve both physiological differences and psychological factors, or even experience with previous treatments such as acupuncture. What's more, diversities in the choice of acupuncture points used, types of stimulation given by acupuncturists, and the perioperative timings could affect reproducibility of experiments conducted and thus cause problems for making standardized rules on the use of AA.

There are still practical obstacles to overcome. For AA to be used effectively in hospitals, there needs to be good teamwork between anesthetists, surgeons, acupuncturists, nursing staff, and physiotherapists, etc. However, many people have not had enough training to work together properly. Standardized perioperative workflows for AA are also lacking. Finally, some hospitals do not provide sufficient support. In addition, different health systems, reimbursement rules, and legislative frameworks in different areas may influence the feasibility and sustainability of AA perioperative care. This problem could be more serious in environments where acupuncture is not included as part of perioperative care.

9 FUTURE PERSPECTIVES

In order to further strengthen the evidential basis supporting the use of AA, there needs to be large-scale, well-designed multicenter trials that adopt common perioperative outcome indicators, including the postoperative pain trajectory, opioid consumption, rehabilitation quality, complication incidence, and hospitalization time [63, 65]. Furthermore, we need to clarify whether "acupuncture anesthesia" refers to intraoperative AA or postoperative acupuncture analgesia, as this may help improve the comparability of results, thereby enabling better summarization of evidence [52, 58].

In clinical practice, AA will probably be accepted as part of multimodal perioperative analgesia instead of replacing conventional anesthesia [66]. AA could be used in ERAS programs, especially in cases requiring fewer opioids and earlier recovery of functions. For better repeatability, standard operating procedures need to include the patient qualification standard, choice of acupoints, stimulation parameters (frequency and intensity), and timing in relation to surgery. In addition, a personalized strategy based on the patients' level of anxiety, pain sensitivity, number of diseases, or previous response to acupuncture may help find out the population who will benefit most from AA [54, 67, 68].

In terms of the system level, the implementation depends on a structured, multidisciplinary training and systematic institutional support. A stratified education programme could facilitate safer integration into daily practice by specifying the role-specific competence of anesthesiologists, acupuncturists, and perioperative nurses involved. Both real-world evidence and health economic evaluation are still required to demonstrate the cost-effectiveness and provide information for reimbursement, as well as for policy-making. By improving the methodology, standardizing the protocol, and selecting patients accurately, AA might evolve as a reliable adjunct for perioperative pain management to support safe analgesia and better rehabilitation.

10 CONCLUSION

This review aims to summarize the function of AA in comprehensive perioperative pain treatment which includes history development, mechanism explanation, real world application, and current challenges. Integrating basic theory of TCM and up-to-date anesthesia science, AA can serve as an innovative non-drug therapy in the current perioperative healthcare scenario [13, 47].

From a mechanistic point of view, AA shows pain relief action and control role via synchronized control over the neurological system, immune system, hormonal system, and autonomic nervous system [69]. Both experimental and clinical studies suggest that AA influences the set up of related biological network which can transmit pain information, generate inflammation reaction and adjust perioperative stress condition [28, 29, 40].

In terms of clinical studies, AA shows great potential as one element of multimodal anesthesia regimens. There seems to be enough evidence to show that AA can reduce the use of anesthetic drugs and analgesics, and alleviate post-operative pain. It also can help patients' speedy recovery and decrease some kinds of anesthetic complications [14, 17, 18]. But we must stress that AA at present just takes on a supplementary role instead of replacing traditional anesthesia method especially when conducting complicated operations or serious surgical invasions [11, 47].

In spite of all these impressive results, there are still quite some significant constraints that ought to be brought up. Studies conducted to date tend to have relatively small samples, insufficient number of high quality randomized controlled trials, and great diversity in acupuncture techniques plus perioperative management methods [13, 46]. In addition, methodological issues such as inadequate blinding and regional concentration of studies may further limit the generalizability of current evi-

dence. Some practical difficulties like differences in practitioner training level, and lack of enough institutional backing will also prevent us from popularizing AA treatment across different hospitals or medical centers.

To address these challenges, future research should focus on the development of standardized intervention protocols, the conduct of large-scale multicenter randomized controlled trials, and the clarification of the distinct roles of intraoperative and postoperative acupuncture interventions. Furthermore, the establishment of structured training programs and interdisciplinary collaboration will be essential for integrating AA into modern perioperative care systems [46].

In summary, integrating traditional Chinese medicine methods into modern perioperative procedures is a significant direction. And with method improvement and further clinical studies verification, AA would have the possibility to become a science-based complementary option for better pain relief and quicker recovery after operation [47].

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Author contributions

Chenlei Qian was responsible for conceptualization, literature search, writing the original draft, and reviewing and editing the manuscript.

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Supplementary Information

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Supplementary Information

Supplementary Table 1. Representative PubMed search strategy

Item	Details
Database	PubMed
Search date	March 1, 2026
Time frame	From database inception to December 31, 2025
Language	English or Chinese (PubMed filters)
Search string	((“acupuncture anesthesia”[Title/Abstract] OR “acupuncture anaesthesia”[Title/Abstract] OR “acupuncture-assisted anesthesia”[Title/Abstract] OR “acupuncture-assisted anaesthesia”[Title/Abstract] OR electroacupuncture[Title/Abstract] OR “electro-acupuncture”[Title/Abstract] OR “acupuncture analgesia”[Title/Abstract] OR “acupuncture”[MeSH Terms]) AND (perioperative[Title/Abstract] OR intraoperative[Title/Abstract] OR postoperative[Title/Abstract] OR anesthesia[Title/Abstract] OR anaesthesia[Title/Abstract] OR analgesia[Title/Abstract] OR “pain management”[Title/Abstract] OR “enhanced recovery”[Title/Abstract] OR ERAS[Title/Abstract] OR “surgical procedures, operative”[MeSH Terms]))

Note: ERAS, enhanced recovery after surgery.

Perioperative Precision Medicine

Zentime

PERSPECTIVE

From organ preservation to xenotransplantation: Technological pathways toward sustainable organ replacement

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1 INTRODUCTION

Organ transplantation is widely recognized as the most effective therapy for patients with end-stage organ failure. Over the past decades, advances in surgical techniques and immunosuppressive therapies have significantly improved graft survival and patient outcomes. Nevertheless, the global shortage of donor organs remains a critical challenge in transplantation medicine. The demand for transplantable organs far exceeds the available supply, leading to prolonged waiting lists and increased mortality among patients awaiting transplantation.

To address this challenge, research in transplantation medicine has evolved along two main directions. One strategy focuses on improving organ preservation and graft viability through advanced techniques such as machine perfusion. These approaches enable metabolic support and functional assessment of donor organs prior to transplantation and have been shown to enhance graft utilization and transplant outcomes [1, 2].

Another promising strategy seeks to expand the donor pool through xenotransplantation. With the development of gene-editing technologies, particularly clustered regularly interspaced short palindromic repeats-based approaches, genetically modified porcine organs with reduced immunogenicity have now been generated. These advances suggest that xenotransplantation may become a viable strategy to address the global shortage of transplantable organs [3, 4].

In this perspective article, we examine the technological transition from conventional organ preservation methods to xenotransplantation. We highlight the scientific progress achieved in recent years, discuss the major translational barriers that remain, and consider the ethical and regulatory challenges associated with this emerging field.

2 ADVANCES IN ORGAN PRESERVATION TECHNOLOGIES

2.1 Limitations of conventional organ preservation

Static cold storage (SCS) has long been the standard method for preserving donor organs due to its simplicity and wide accessibility. However, SCS does not fully prevent ischemia–reperfusion injury, which remains one of major causes of delayed graft function and early graft failure after transplantation [5].

These limitations become more evident when marginal organs or extended-criteria donors are used. As transplant programs increasingly rely on such donors to expand the organ pool, preservation-related injury has become a critical factor affecting transplant outcomes. Therefore, improving preservation technologies is essential not only for maintaining graft quality but also for safely increasing organ utilization.

2.2 Machine perfusion as a bridge technology

Machine perfusion technologies have significantly transformed the concept of organ preservation. Unlike SCS, perfusion-based preservation provides continuous oxygen and nutrients to the organ, allowing metabolic activity to be sustained and graft viability to be assessed prior to transplantation.

Clinical studies have demonstrated that ex vivo perfusion can increase the utilization of donor hearts and reduce the incidence of severe primary graft dysfunction after transplantation [1]. Similarly, ischemia-free liver transplantation using continuous perfusion has been shown to improve control of ischemia–reperfusion injury and achieve better postoperative graft function compared with conventional preservation methods [2].

These technological advances suggest that machine perfusion not only improves current transplantation practice but also provides a potential platform for future innovations. In particular, perfusion systems may enable targeted therapies, immune modulation, or functional optimization of donor organs prior to implantation.

Importantly, the development of machine perfusion technologies also provides a technical foundation for future xenotransplantation research. Controlled perfusion environments could facilitate graft evaluation and immune conditioning before transplantation, thereby bridging current allotransplantation techniques and emerging xenotransplantation strategies.

3 XENOTRANSPLANTATION AS A FUTURE STRATEGY

3.1 Gene editing and donor modification

Xenotransplantation has long been proposed as a potential solution to the shortage of human donor organs. Among possible donor species, pigs are considered the most suitable candidates due to their physiological compatibility with humans and the feasibility of large-scale breeding.

Historically, however, xenotransplantation faced strong immune barriers posed by carbohydrate antigens expressed on porcine endothelial cells. Recent advances in gene-editing technologies, particularly clustered regularly interspaced short palindromic repeats-based approaches, have made it possible to remove key xenoantigens and introduce human regulatory genes into donor animals. These genetic modifications significantly reduce the immunogenicity of porcine organs and improve graft survival in experimental models [6].

Preclinical studies using multi-gene-edited pigs have demonstrated prolonged graft survival and improved compatibility between donor organs and recipients [4, 6]. These findings suggest that xenotransplantation may become an important strategy for expanding the donor pool in the future.

3.2 Immunological barriers

Despite these advances, immune rejection remains the central challenge in xenotransplantation. Xenograft rejection can occur at several stages, including hyperacute rejection, acute vascular rejection, and chronic graft injury.

Hyperacute rejection occurs within minutes to hours after transplantation and is primarily mediated by pre-existing natural antibodies that recognize carbohydrate antigens on porcine endothelial cells. This interaction triggers complement activation, endothelial injury, and rapid graft failure [7].

Acute rejection develops over days or weeks and involves both humoral and cellular immune responses. Antibody-mediated injury leads to complement deposition and vascular damage, while immune cells—including macrophages, natural killer cells, and T lymphocytes—contribute to inflammatory graft injury [8, 9].

A schematic overview of these immune mechanisms and the gene-editing strategies used to mitigate them is presented in **Figure 1**.

4 TRANSLATIONAL MODELS AND CLINICAL PERSPECTIVES

Animal models are essential for bridging experimental research and clinical application in xenotransplantation. Pig-to-nonhuman primate transplantation models are widely used to investigate graft survival, immune responses, and systemic complications associated with xenografts.

Recent studies using genetically modified pigs have demonstrated significant improvements in xenograft survival in preclinical models [6, 8]. These experiments indicate that combining genetic engineering of donor animals with optimized immunosuppressive strategies can substantially reduce early immune injury.

Although these findings are encouraging, additional studies are required to evaluate long-term graft function, immune compatibility, and safety before xenotransplantation can be widely applied in clinical practice.

The major technological advances discussed in this article are summarized in Supplementary Table 1.

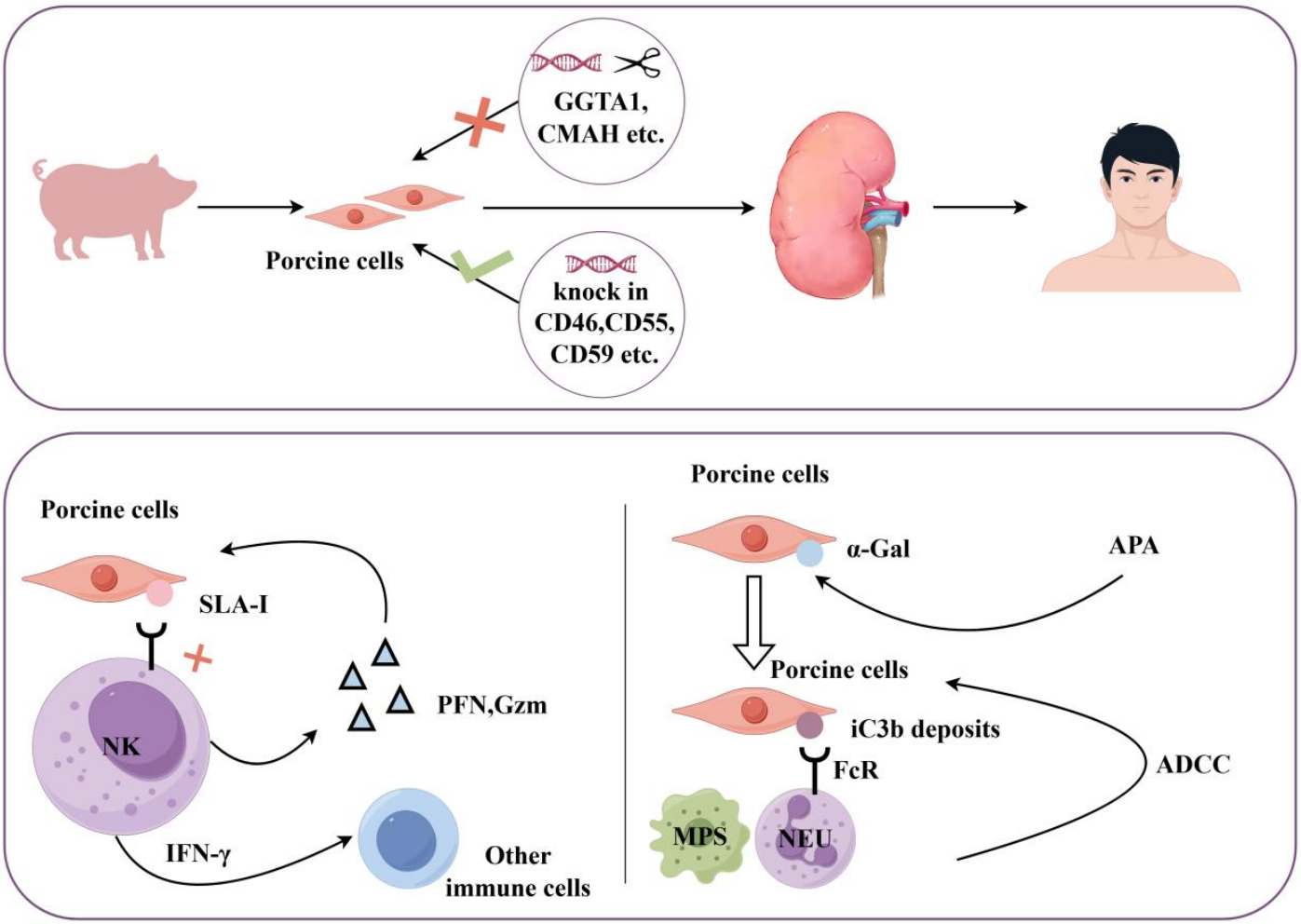

Figure 1. Immune barriers and gene-editing strategies in xenotransplantation. Gene-editing technologies enable the deletion of major porcine xenoantigens (e.g., GGTA1, CMAH) and the introduction of human regulatory genes (e.g., CD46, CD55, CD59) to reduce complement-mediated injury and immune rejection. The schematic illustrates the main immune pathways involved in xenograft rejection—including humoral responses (complement activation, natural killer cells) cellular responses (macrophages, neutrophils)—as well as the molecular strategies used to improve graft compatibility. Created with BioRender.com. GGTA1, α-1,3-galactosyltransferase; CMAH, CMP-N-acetylneuraminic acid hydroxylase; CD46, membrane cofactor protein; CD55, decay-accelerating factor; CD59, membrane attack complex inhibitory protein; SLA-I, swine leukocyte antigen class I; NK, natural killer cell; PFN, perforin; Gzm, granzyme; IFN-γ, interferon-gamma; α-Gal, galactose-α-1,3-galactose; APA, alternative pathway of complement activation; iC3b, inactive complement component 3b; FcR, Fc receptor; MPS, mononuclear phagocyte system; NEU, neutrophil; ADCC, antibody-dependent cell-mediated cytotoxicity.

5 ETHICAL AND BIOSAFETY CONSIDERATIONS

The development of xenotransplantation raises several important ethical and biosafety questions. One major concern is the welfare of donor animals. The production of genetically modified pigs involves breeding, genetic manipulation, and controlled housing conditions, all of which must follow strict ethical guidelines to ensure humane treatment.

Another important issue is the potential risk of zoonotic infection. Porcine endogenous retroviruses and other porcine microorganisms may theoretically be transmitted to human recipients. Although gene-editing technologies and pathogen-free breeding programs have significantly reduced these risks, long-term monitoring of recipients remains essential [10].

Furthermore, the future implementation of xenotransplantation will depend on public acceptance and transparent regulatory frameworks. Policies addressing donor animal welfare, recipient monitoring, and equitable access to transplantation technologies will be critical for the responsible development of this field.

6 CONCLUSION

Organ transplantation is entering a new era in which advances in organ preservation and the search for alternative donor

sources are becoming increasingly interconnected. Machine perfusion technologies have already improved the preservation and evaluation of donor organs, while xenotransplantation offers a potential long-term solution to the global shortage of transplantable organs.

Nevertheless, several scientific and ethical challenges remain. Immune incompatibility, coagulation disturbances, zoonotic infection risks, and regulatory considerations must all be addressed before xenotransplantation can become a routine clinical therapy.

Future progress will require close collaboration across multiple disciplines, including transplantation surgery, immunology, genetic engineering, and bioethics. Through continued technological innovation and responsible governance, xenotransplantation may ultimately provide a sustainable solution for patients suffering from end-stage organ failure.

DECLARATIONS

Author contributions

Lu Cheng conceived the manuscript, wrote the original draft, and approved the final version.

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Data availability

Data sharing is not applicable to this article, as no datasets were generated or analyzed during the current study. All information is derived from publicly available articles and datasets.

Ethics approval and consent to participate

Not applicable. This manuscript does not contain any studies involving human participants or animals performed by any of the authors.

Consent for publication

Not applicable. This manuscript does not include any details, images, or videos relating to an individual person.

Competing interests

The author declares no competing interests.

Acknowledgements

Not applicable.

Supplementary Information

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Supplementary Information

Supplementary Table 1. Representative technological advances from conventional organ preservation to xenotransplantation

Technology/strategy	Main application	Major advantage	Current limitation	References
Static cold storage (SCS)	Conventional organ preservation	Simple and widely used	Limited protection against ischemia–reperfusion injury	[1, 2]
Hypothermic oxygenated perfusion (HOPE)	Kidney and liver preservation	Reduces ischemic and mitochondrial injury	Clinical benefits vary across donor settings	[3, 4]
Normothermic machine perfusion (NMP)	Kidney, liver, and heart preservation	Ennctional assessment and organ reconditioning	Cost, complexity, and standardization remain issues	[5-7]
Targeted immunomodulation	Desensitization and rejection control	Improves immune compatibility and graft survival	Long - term balance between efficacy and safety remains unresolved	[8-10]
Multi-gene-edited porcine donor organs	Xenotransplantation	Expands potential donor pool and reduces major xenoantigenicity	Residual immune, coagulation, and biosafety barriers persist	[11-13]

Note: This table summarizes representative technological advances in organ transplantation and xenotransplantation discussed in the main text.

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Perioperative Precision Medicine

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REVIEW ARTICLE

Dual roles of sphingosine-1-phosphate receptor 3 in inflammation, ischemia-reperfusion injury, and vascular homeostasis

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Abstract

Sphingosine-1-phosphate receptor 3 (S1PR3) is a member of the G protein-coupled receptor (GPCR) family, structurally characterized by seven transmembrane domains. It exhibits diverse biological functions by binding to three major Gα protein subtypes (Gi/o, Gq, and G12/13), thereby activating downstream signaling pathways such as phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt), phospholipase C/calcium (PLC/Ca^{2+}), and Ras homolog family member A/Rho-associated coiled-coil containing protein kinase (RhoA/ROCK). S1PR3 displays tissue- and cell-specific expression patterns, with high levels observed in the cardiovascular system (endothelial cells, vascular smooth muscle cells, cardiac fibroblasts), immune system (macrophages, dendritic cells), nervous system (astrocytes, microglia), and liver (hepatocytes, hepatic stellate cells). This expression profile enables S1PR3 to play pivotal roles in regulating inflammation, maintaining vascular homeostasis, repairing liver damage, protecting neural function, and preserving immune balance. Under pathological conditions, S1PR3 exhibits dual functionality. At physiological concentrations or under mild pathological conditions, it exerts protective effects—for instance, preventing cardiomyocyte and hepatocyte apoptosis and maintaining blood-brain barrier (BBB) integrity. Conversely, overactivation leads to tissue damage, including exacerbated inflammatory infiltration (e.g., in acute respiratory distress syndrome, ARDS), promotion of fibrosis (e.g., hepatic fibrosis), and vascular injury (e.g., atherosclerosis). S1PR3 has emerged as a promising therapeutic target for cardiovascular diseases, neurological injuries, and liver disorders. However, clinical application of S1PR3 modulators is hindered by off-target effects (e.g., bradycardia due to insufficient subtype selectivity) and risks of immunosuppression, underscoring the need for more selective ligands and personalized therapeutic regimens.

Keywords: Sphingosine-1-phosphate receptor 3, Sepsis, Liver injury, Inflammation

Highlights

- Clarifies S1PR3's dual functionality, which shifts depending on S1P concentration, injury stage, and cell type—resolving long-standing controversies in the field.
- Bridges basic research and clinical translation by defining S1PR3's roles in diseases like sepsis, ARDS, and liver fibrosis, laying a solid foundation for its development as a potential therapeutic target.
- Deciphers the complex regulatory network involving S1PR3, CXCR1, PAR1, and the NF-κB pathway; identifies the key role of S1PR3/CXCR1 heterodimers in neutrophils of pneumonia patients as a major breakthrough; and addresses current limitations while proposing targeted future research strategies.

1 BASIC CHARACTERISTICS AND DISTRIBUTION OF SPHINGOSINE-1-PHOSPHATE RECEPTOR 3 (S1PR3)

Sepsis is a life-threatening systemic inflammatory response syndrome triggered by infection, characterized by a dysregulated host immune response that often leads to progressive immune dysfunction, organ damage, and even death [1]. Sphingosine-1-phosphate receptors (S1PRs) are an important type of non-Toll-like receptors (non-TLRs) belonging to the G protein-coupled receptor (GPCR) family. They bind to ligands like sphingosine-1-phosphate (S1P), initiating intracellular transmembrane signal transduction, promoting activation of G protein downstream signaling pathways, and enabling diverse biological functions, including regulation of cell proliferation, inflammatory responses, and angiogenesis [2].

Five S1PR subtypes (S1PR1 to S1PR5) have been identified, among which S1PR1, S1PR2, and S1PR3 are ubiquitously expressed. In recent years, S1PR3 has garnered increasing research attention. It is highly expressed in macrophages and can couple with three types of G proteins (Gi, Gq, and G12/13), participating in various pathophysiological processes by regulating oxidative stress responses [3, 4]. Specifically, S1PR3 activates downstream signaling pathways, including PI3K/Akt, phospholipase C (PLC), Rho family GTPases (Rac and Rho), adenylyl cyclase, Jun N-terminal kinase (JNK), and mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK), thereby exerting its biological effects [5].

2 S1PR3 AND INFLAMMATORY RESPONSE

S1P is a bioactive signaling molecule derived from mammalian membrane sphingolipids, functioning in immune and inflammatory responses as well as the cardiovascular system [2]. Most of S1P's biological functions are mediated by S1PR1 to S1PR5. Among these, S1PR3 participates in numerous biological activities, including inflammation and vascular barrier function [6-8]. S1PR3 promotes myeloid differentiation at the expense of other cell lineages (especially erythropoiesis) and enhances the survival of long-term hematopoietic stem cells (LT-HSCs), closely resembling tumor necrosis factor alpha (TNF-α)-induced emergency hematopoiesis [9]. Thus, S1PR3 acts as an enhancer of TNF-α, utilizing the NF-κB inflammatory signaling pathway to promote HSC survival and myeloid differentiation.

Although TNF-α levels increase with aging and in myeloid malignancies, the mechanisms by which this inflammatory environment induces leukemia and the specific cell subtypes prone to transformation remain unknown [10-12]. Recent studies have revealed that acute TNF-α treatment upregulates S1PR3 membrane levels in primary acute myeloid leukemia (AML) cell lines, while S1PR3 antagonism counteracts TNF-α-induced myeloid differentiation [13]. Previous research demonstrated that S1PR3 overexpression (S1PR3OE) driven by the lysosomal M promoter—rather than the F4/80 promoter—induced leukemic transformation in mice, suggesting that the initiating cell originates from primitive subsets rather than the mature myeloid lineage [13]. However, S1PR3OE in human HSCs did not cause de novo AML in xenografts, indicating that other factors such as the microenvironment and species differences must be considered [14].

S1PR3 expression is elevated in circulating neutrophils of patients with sepsis and pneumonia, forming heterodimers with chemokine (C-X-C motif) receptor 1 (CXCR1), a biomarker of inflammation also known as interleukin-8 (IL-8) receptor alpha (IL-8RA) [15, 16]. This positions S1PR3 as a molecular target for sepsis-associated lung injury.

Furthermore, in vivo studies on S1PR3-mutant mice revealed that protease-activated receptor 1 (PAR1) amplifies inflammation through sphingosine kinase 1 (SphK1)-S1PR3 signaling crosstalk, promoting disseminated intravascular coagulation and death [3]. Endothelial microparticles—complex vesicular structures shed by activated or apoptotic endothelial cells (ECs)—contain enzymes, transcription factors, and mRNA, and are released in response to inflammatory stimuli such as TNF-α, other cytokines, lipopolysaccharide (LPS), and thrombin [17-19]. Studies have shown that EC-damaging substances may induce nitration and shedding of EC S1PR3 into microparticles [20]. Our experiments further demonstrated that S1PR3-containing microparticles enhance EC barrier disruption in vitro, consistent with their role in sickle cell disease, suggesting that microparticles serve as both biomarkers of EC damage and cellular sources of inducers for vascular dysfunction [20].

Table 1. S1PR3-related core signaling pathways and their biological functions

Category	Key information	Related references
S1P basic characteristics & functions	Sphingosine-1-phosphate (S1P) (from membrane sphingolipids): acts in immunity, inflammation, cardiovascular system; functions mediated by S1PR1-S1PR5.	[2]
S1PR3 core biological functions	(1) Inflammation, vascular barrier, myeloid differentiation, long-term hematopoietic stem cells (LT-HSCs) survival; (2) Myeloid differentiation (erythropoiesis impaired); (3) Enhances tumor necrosis factor-α (TNF-α) via nuclear factor-κB (NF-κB): supports hematopoietic stem cells (HSCs) survival/differentiation; (4) Heterodimerizes with C-X-C chemokine receptor type 1 (CXCR1) in neutrophils.	[6-9, 15]
S1PR3 in hematopoiesis & leukemia	(1) Human HSCs S1PR3 mimics murine TNF-α-NF-κB; (2) TNF-α upregulates S1PR3 in AML; antagonism reverses differentiation; (3) Murine S1PR3OE (lysosomal M promoter) induces leukemia; (4) Human S1PR3OE no de novo acute myeloid leukemia (AML) in xenografts (species/microenvironment).	[9, 13, 14]
S1PR3 in inflammatory signaling crosstalk	(1) Protease-activated receptor 1 (PAR1) amplifies inflammation via sphingosine kinase 1–sphingosine-1-phosphate receptor 3 (SphK1–S1PR3) (murine): promotes coagulation/death; (2) TNF-α/thrombin: EC S1PR3 nitration, shedding into microparticles.	[17, 18, 21]
S1PR3-containing endothelial microparticles	(1) EC microparticles (activated/apoptotic ECs) carry S1PR3; released by TNF-α/lipopolysaccharide (LPS)/thrombin; (2) S1PR3-microparticles disrupt EC barrier (consistent with sickle cell disease); (3) LPS+cytokines boost EC microparticles; high levels link to thrombosis/dysfunction.	[17-22]
S1PR3 in disease-related expression & target value	(1) S1PR3 high in sepsis/pneumonia neutrophils; (2) CXCR1 (interleukin-8 receptor α (IL-8RA), inflammation marker) binds S1PR3; S1PR3 is a target for sepsis lung injury.	[15, 16]
Unclear research directions	(1) TNF-α-enriched inflammation: leukemia induction mechanism/cell subtypes; (2) S1PR3-microparticles: EC barrier disruption mechanism; (3) Functional differences: nitrated vs non-nitrated S1PR3.	[10-12, 20]

Note: S1PR3, Sphingosine-1-phosphate receptor 3; GPCR, G protein-coupled receptor; PKC, Protein kinase C; CXCR1, C-X-C chemokine receptor type 1; CXCL8, C-X-C motif chemokine ligand 8; IRI, Ischaemia–reperfusion injury; CXCL12, C-X-C motif chemokine ligand 12; DARC, Duffy antigen receptor for chemokines; ELR+, Glutamic acid-leucine-arginine positive; TNF-α, Tumour necrosis factor-α; β2AR, Beta-2 adrenergic receptor.

Combined LPS and cytokine treatment increases EC microparticles production [21]. Microparticles are detectable in plasma of healthy individuals, and higher concentrations under pathological conditions are linked to increased thrombotic risk and endothelial dysfunction (ED) [22]. Related signaling pathways are summarized in **Table 1**.

3 S1PR3 AND HEPATOCELLULAR INJURY

3.1 Hepatic fibros

Hepatic fibros is a common consequence of various liver injuries, including viral infection, drug abuse, and alcohol intoxication. After liver injury, levels of SphK1 and S1P increase substantially. In vitro studies have shown that S1PR1/3 mediate S1P-triggered migration of human HSCs and activation of fibrosis [23]. Blockade of S1PR1/3 significantly reduces pathological angiogenesis and hepatic fibrosis in mice with bile duct ligation (BDL) [24]. As hepatic fibrosis progresses, S1P promotes bone marrow mesenchymal stem cell (BMSC) migration via S1PR3. Suramin markedly reduces the number of bone marrow-derived cells during cholestasis, suggesting that the S1P/S1PR3 signaling pathway significantly affects cholestatic hepatic fibrosis by facilitating bone marrow cell homing [25]. Additionally, S1PR1 and S1PR3 are essential for transforming growth factor-β1 (TGF-β1)-induced transdifferentiation of BMSCs into hepatic myofibroblasts [26].

The RNA-binding protein HuR regulates tumor cell migration. HuR mRNA levels are elevated in hepatic fibrotic tissues from humans and mice. In vitro experiments confirmed that the S1PR3/p38 signaling pathway promotes BMSC migration by inducing HuR phosphorylation and cytoplasmic translocation [27]. Furthermore, the S1PR2/3/Gi/PI3K/Rac1 signaling pathway participates in the migration and recruitment of bone marrow mesenchymal monocytes. Administration of the S1PR2 antagonist JTE-013 or the S1PR3 antagonist CAY-10444 significantly reduces recruitment of these monocytes to fibrotic livers, alleviating inflammation and fibrosis in BDL mice [28]. Palmitic acid induces S1P formation in hepatocellular carcinoma cells and promotes its extracellular release. S1P participates in HSC fibrosis via S1PR3. Conditioned medium from hepatocellular carcinoma cells upregulates α-smooth muscle actin (α-SMA) expression in HSCs in an S1P-dependent manner, initiating hepatic fibrosis [29]. Recent studies have shown elevated S1PR3 levels in patients with endometriosis, and S1PR3 participates in TGF-β1-induced fibrosis of endometrial adenocarcinoma cells [29, 30].

Our study provides spatial (zonal) characterization of liver sinusoidal endothelial cells (LSECs; zones 1–3), identification

of transcriptomic changes associated with liver cirrhosis, and demonstration of relationships between these transcriptomic changes and phenotypic alterations. Zone 3 LSECs are most susceptible to cirrhosis-associated damage, exhibiting increased capillarization and decreased endocytic capacity. Identification of the most dysfunctional LSEC populations will be valuable for developing effective therapeutic strategies. Furthermore, CD34 is a more useful marker of LSEC capillarization in liver cirrhosis than CD31 [31].

With recent advances in molecular and cell biology technologies, understanding of the pathogenesis of portal vein thrombosis (PVT) associated with liver cirrhosis has deepened. PVT occurrence involves multiple factors, including local and systemic inflammatory responses, disturbances in coagulation-fibrinolysis balance, endothelial dysfunction, and alterations in gut flora [32-34]. The crosstalk among these molecular and cellular mechanisms renders PVT pathogenesis a complex process.

Under cirrhotic conditions, changes in the hepatic microenvironment form an intricate network with systemic immune disorders and coagulation system imbalances. Elucidating the functional network underlying PVT occurrence not only clarifies its pathogenesis but also informs early diagnosis and personalized treatment. This article reviews literature from the past five years, discussing relationships between key factors (inflammatory response, coagulation-fibrinolysis balance, endothelial dysfunction, gut flora) and PVT formation in cirrhotic patients, aiming to provide theoretical support for improving patient management and prognosis [35].

3.2 Regulatory role of S1P and its receptors in sepsis-induced liver injury

S1P and its receptors play key roles in immune and metabolic processes, closely associated with various inflammatory responses, oxidative stress, and lipid deposition diseases. As an integral organ for immunity and metabolism, the liver not only defends against bacterial toxins during sepsis but also regulates metabolism of deposited substances post-injury. S1P and S1PR1 to S1PR3 are highly expressed in the liver and act through multiple signaling pathways. For instance, the spinster homolog 2 (Spns2)/S1P signaling pathway exerts inhibitory effects during early inflammatory responses to prevent cytokine storms and promotes later-stage effects to reduce immunosuppression-induced damage [36]. Additionally, S1P likely plays an essential role in bile acid metabolism regulation, particularly relevant in liver injury. Further research on mutual regulation among liver injury, immunity, and metabolism may facilitate discovery of novel therapeutic targets [37]. S1PR-targeted drugs have shown efficacy in regulating immunity, and future studies should explore new metabolic targets to advance treatment strategies for inflammatory and metabolic disturbances associated with sepsis-induced liver injury [38].

3.3 Expression of markers and functional specificity of LSECs versus portal vein endothelial cells

Endothelial cell cultures are valuable tools for investigating liver physiology and pathophysiology in vitro. However, the marked heterogeneity of endothelial cells within and between organs, combined with the tendency for cultured cells to lose tissue-specific markers, complicates such studies. Although all endothelial cells share certain characteristic features, specific markers or marker combinations are needed to define distinct endothelial populations. To date, the study of human sinusoidal endothelial cells (HSECs) in vitro has been hampered by the lack of specific markers that conclusively identify these cells and discriminate them from vascular or lymphatic endothelial cells. This remains partially true, as many classic endothelial markers are widely expressed, and considerable variability exists in phenotypic marker detection between animal and human systems (e.g., CD31 and von Willebrand factor). Currently, no single molecule is known to be expressed exclusively on hepatic sinusoidal endothelium. However, increasing knowledge of endothelial receptors is providing a larger, better-defined set of phenotypic markers. In addition to their utility in phenotyping or sorting specific endothelial cells for culture, tissue-specific receptors offer clues to the functions of the cells under study, exemplified by the numerous scavenger receptors expressed on HSECs.

Very low-passage cells retain fenestrations in vitro for a short time, but these rapidly disappear within one or two passages, as does expression of vascular adhesion protein-1 (VAP-1) [39-41]. Apart from these changes, the cells remain relatively phenotypically stable for 7–8 passages and can be identified by expression of CD31, lymphatic vessel endothelial hyaluronan receptor 1 (LYVE-1), dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin-related (L-SIGN), Stabilin-1, and lack of CD34 and prospero homeobox 1 (PROX-1) [39, 42]. These markers confirm endothelial identity while excluding vascular and lymphatic endothelial contamination and, together with markers excluding leukocyte origin, can confirm the sinusoidal nature of the cells [38].

4 EFFECTS OF S1PR3 ON VASCULAR FUNCTION

Cardiovascular diseases are leading causes of death worldwide. Endothelial cells, vascular smooth muscle cells, cardiomyocytes, and cardiac fibroblasts all express S1PR1–S1PR3, but expression patterns differ significantly. Endothelial cells primarily express S1PR1 and S1PR3, with S1PR1 being most abundant. Vascular smooth muscle cells highly express S1PR2 and S1PR3. Cardiomyocytes predominantly express S1PR1, followed by S1PR3 at approximately half the level. Cardiac fibroblasts express S1PR3 at much higher levels than S1PR1 and S1PR2 [43].

4.1 Regulation of angiogenesis

Walter et al. discovered that S1PR3 knockout mice exhibit impaired angiogenesis. Conversely, S1P or fingolimod (FTY720) activates Src kinase and C-X-C chemokine receptor type 4 (CXCR4)-dependent Janus kinase 2 (JAK2) signaling via S1PR3, promoting angiogenesis in endothelial progenitor cells [44].

In vitro experiments demonstrated that S1P triggers activation of the S1PR3/PI3K/Akt signaling pathway. Under low-concentration S1P stimulation (100 nM), S1PR3 in endothelial progenitor cells primarily mediates Gi protein-dependent PI3K/Akt pathway activation, thereby promoting cell proliferation and inhibiting apoptosis [45].

High-density lipoprotein (HDL)/S1P activates vascular endothelial growth factor receptor 2 (VEGFR2) through S1PR3, promoting proliferation of human umbilical vein endothelial cells and angiogenesis [46]. Li et al. found that S1P/S1PR3 facilitates transdifferentiation of epicardial progenitor cells into smooth muscle-like cells [47]. Recently, Yasuda et al. demonstrated that S1P/S1PR3 promotes corneal angiogenesis by increasing vascular endothelial growth factor A (VEGF-A) production in mice [48].

4.2 Regulation of vascular barrier function

S1PR3 is highly expressed in patients with acute lung injury and severe falciparum malaria complicated by pulmonary edema, primarily in pulmonary endothelial cells and plasma, serving as a potential biomarker for acute lung injury [49]. In LPS-induced acute inflammatory lung injury mouse models, Sammani et al. found that intratracheal administration of high-dose S1P (final plasma concentration ~1 mM) significantly disrupted the alveolar-capillary barrier, an effect prevented by S1PR3 silencing [50]. Different S1P concentrations exert opposing effects on the endothelial barrier: physiological concentrations (10 nM–2 μM) protect the endothelial barrier via the Gi/Rac signaling pathway, whereas higher concentrations activate the S1PR3/RhoA/ROCK pathway, increasing vascular permeability—an effect that may completely counteract the barrier-protective Gi/Rac pathway [51, 52]. Therefore, reducing S1PR3 expression and activation may be an effective strategy for preventing inflammation, acute lung injury, and other diseases linked to endothelial barrier dysfunction [53].

4.3 Regulation of vascular tone

Activating S1PR3 in endothelial cells promotes nitric oxide (NO) production and induces vasorelaxation. In contrast, S1PR3 in vascular smooth muscle cells induces vasoconstriction by increasing intracellular calcium concentration and activating Rho [54]. HDL/S1P promotes calcium mobilization and Gi/PI3K/Akt signaling activation in endothelial cells through S1PR3, facilitating endothelial nitric oxide synthase (eNOS) phosphorylation and ultimately leading to vasodilation [55, 56]. However, other studies have shown that S1P causes vasoconstriction in rat coronary arteries and mouse mesenteric arteries, an effect significantly reduced by pretreatment with the S1PR3-specific antagonist TY52156 [57, 58]. Thus, the effect of S1PR3 activation on vascular tone is complex and requires further detailed investigation.

4.4 Ischemia-reperfusion injury

Accumulating evidence indicates that S1PR3 signaling plays a key role in protecting the heart from ischemia-reperfusion injury (IRI). Administration of HDL/S1P or pretreatment with a specific S1PR3 agonist reduces myocardial infarct size following IRI. Both in vitro and in vivo experiments demonstrate that S1PR3-mediated Akt- and NO-dependent pathways protect cardiomyocytes from apoptosis [52-54]. RhoA has also been implicated in cardioprotection. The G12/13/RhoA/ROCK signaling pathway triggers MRTF-A activation and increases stromal cell protein CCN1 expression, thereby protecting cardiomyocytes from ischemic injury [59-63]. Connexin 43 (Cx43) is a major component of ventricular gap junctions, which transmit action potentials and bioactive substances. Recent studies have shown that S1P regulates Cx43 phosphorylation, expression, and distribution via S1PR2/3, ultimately reducing myocardial cell death and sudden cardiac death caused by IRI [64]. Cx43 uncouplers (e.g., heptanol and Gap26) lower IRI-induced mortality mediated by S1PR2/3 [65]. Interestingly, Wafa et al. found that in the early stage of acute coronary syndrome, S1P/S1PR3 induces coronary artery constriction and impairs myocardial contractile function, although S1PR3-induced vasoconstriction may be counteracted by S1PR2-mediated vasorelaxation [53-66]. Thus, S1PR3 activation may represent an important strategy for preventing myocardial IRI.

Studies have demonstrated that S1PR3 participates in inflammation, proliferation, migration, tumor invasion, IRI, tissue fibrosis, and vascular activity [67]. In a mouse model of myocardial IRI, HDL and its component S1P protect the heart via an S1PR3-mediated signaling pathway [68]. S1PR3(–/–) mice are protected from renal IRI through mechanisms involving bone marrow-derived dendritic cells (BMDCs) and their immunomodulatory functions [69]. Adoptive transfer of S1PR3(–/–) BMDCs prevents renal IRI through splenic interactions and expansion of splenic CD4+Foxp3+ regulatory T cells. In contrast to its protective role in cardiac IRI, S1PR3 exerts opposite effects in renal IRI, likely due to differences in disease stage and cell/tissue contexts.

S1PR1 and S1PR2 have been implicated in microglial activation during cerebral IRI [70-72]. A recent study showed that S1PR3 contributes to microglial activation and M1 macrophage polarization in a mouse brain IRI model [73]. Our study

demonstrated that S1PR3 inhibition reduces IRI, confirmed by reduced infarct volume. H&E, Nissl, and Fluoro-Jade C staining confirmed that S1PR3 mediates brain damage during cerebral IRI [74].

Neuronal nitric oxide synthase (nNOS) mediates early neurological damage, with nNOS overexpression playing a key role in early ischemia and excitotoxic injury [75]. Inducible NOS (iNOS) subsequently increases, and both adversely affect cerebral ischemia. NO production, catalyzed by NOS from L-arginine and oxygen, can react with superoxide to form peroxynitrite, a potent oxidant [76]. During ischemia, NO produced by nNOS and iNOS may be neurotoxic, partly due to peroxynitrite free radical formation causing direct damage to mitochondrial enzymes and DNA [77]. Additionally, increased NO production from nNOS or iNOS promotes ischemic damage through free radical injury, tissue inflammation, and microcirculatory failure [78]. nNOS knockout mice exhibit smaller infarct sizes and fewer neurological deficits after middle cerebral artery occlusion [79]. iNOS knockout mice also show fewer neurological deficits and reduced infarct volumes [80]. In our experiment, nNOS and iNOS expressions were elevated following cerebral ischemia compared with sham-operated controls, consistent with previous studies. S1PR3 inhibition reduced nNOS expression and NO content. Heo and Im found that S1PR3 inhibition reduces LPS-induced inflammatory gene expression, including iNOS and cyclooxygenase-2 [74]. However, our study found that S1PR3 inhibition did not reduce iNOS expression, suggesting that S1PR3 reduces NO primarily by decreasing nNOS rather than iNOS.

4.5 Atherosclerosis

Atherosclerosis is a chronic inflammatory disease, with ED being a key driver of its development. HDL is considered atheroprotective. Kimura et al. found that HDL activates PI3K/Akt, p38, and Rho/ROCK pathways via S1P/S1PR3, promoting endothelial cell migration and exerting anti-atherosclerotic effects [81]. Additionally, S1P prevents macrophage apoptosis by activating the S1PR3/STAT3/surviving signaling pathway, reducing the risk of plaque rupture in advanced atherosclerotic lesions [82].

Macrophages with S1PR3 knockout are resistant to 22(R)-hydroxycholesterol-stimulated cholesterol efflux. Conversely, the S1P/S1PR3/Gq/PLC/protein kinase C (PKC) signaling pathway may promote cholesterol efflux by phosphorylating and stabilizing the ATP-binding cassette transporter A1 (ABCA1) protein [83]. A recent study emphasized the core role of ED as a major precursor of inflammation, not only accelerating atherosclerosis progression but also causing plaque instability. Hallmarks of ED include reduced NO production/availability and endothelial cells exhibiting an inflammatory phenotype. The endothelial glycocalyx, a gel-like structure composed of glycoproteins and extracellular matrix components, participates in transendothelial transport. Endothelial glycocalyx damage impairs eNOS expression, leading to reduced NO availability and production [84].

Various inflammatory signals activate endothelial cells, triggering upregulation of cell adhesion molecules, including pro-inflammatory E-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1). Leukocyte diapedesis occurs, accompanied by increased endothelial permeability [85]. Activated endothelial cells secrete cytokines and function similarly to macrophages. Dysfunctional, inflamed endothelium facilitates lipoprotein penetration and trapping beneath the endothelial cell layer, where these lipoproteins are subsequently oxidized [66]. Deposited lipoproteins are taken up by macrophages and smooth muscle cells, leading to foam cell formation and fatty streak accumulation. Abnormalities in cell clearance—specifically, failure to properly remove apoptotic endothelial cells—result in vascular sclerosis [86]. Some endothelial cells may undergo endothelial-mesenchymal transition, promoting extracellular matrix deposition and further exacerbating vascular stiffness [87]. Arterial constriction caused by lipid accumulation, combined with impaired vasodilation, expands regions of turbulent or oscillatory blood flow, further intensifying endothelial cell activation and denudation. These regions carry a high risk of rupture for lipid-rich plaques with thin fibrous caps, potentially leading to thrombotic occlusion. Expression of procoagulant factors—tissue factor, plasminogen activator inhibitor-1 (PAI-1), and von Willebrand factor (vWF)—further heightens this risk [84-88]. Thus, ED serves as a precursor to both atherosclerosis and thrombosis.

Patients with coronary artery disease often experience sleep disturbances, reduced melatonin levels, and circadian rhythm misalignment [89]. Sleep disturbances are linked to higher levels of inflammatory markers, ED, and reduced cardiac vagal modulation—all contributing to coronary artery disease development and progression [90]. Relatively few studies have established a clear connection between lifestyle behaviors (e.g., physical activity level, a modifiable cardiovascular risk factor) and endothelial function in patients with cardiovascular disease [91]. In the present study, multiple regression analysis revealed a significant association between low physical activity levels and ED (**Table 1**). Analysis of the control group found that physically active patients had better endothelial function than sedentary ones. Consistent with this, a study involving 2,363 patients with prediabetes and type 2 diabetes showed that sedentary behavior is linked to higher levels of low-grade inflammatory biomarkers (C-reactive protein, soluble ICAM-1, IL-6, and TNF-α) as well as biomarkers of ED, which are key components of the atherosclerotic process illustrated in **Figure 1** [92].

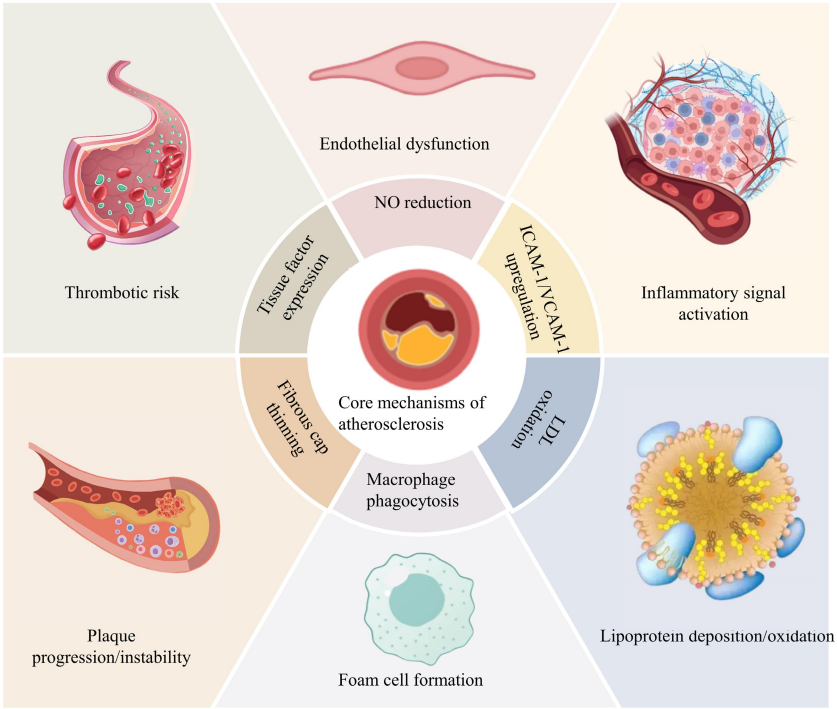

Figure 1. Core mechanisms of atherosclerosis. Binding of sphingosine-1-phosphate (S1P) to S1P receptor 3 (S1PR3) exerts pleiotropic effects in vascular biology, regulating angiogenesis, vascular tone, atherosclerosis, and ischemia-reperfusion injury protection via distinct signaling pathways. This figure depicts the core mechanisms of atherosclerosis, including endothelial dysfunction (manifested as reduced nitric oxide (NO) bioavailability and upregulated intercellular adhesion molecule 1 (ICAM-1)/vascular cell adhesion molecule 1 (VCAM-1)), inflammatory signaling activation, lipoprotein deposition and oxidation (involving low-density lipoprotein (LDL) oxidation), macrophage phagocytosis and subsequent foam cell formation, plaque progression and instability (characterized by fibrous cap thinning and tissue factor expression), as well as heightened thrombotic risk. Created with Microsoft PowerPoint.

5 S1PR3 AND IMMUNE RESPONSE

Growing evidence suggests that S1PR3 regulates the biological functions of certain immune cells, including dendritic cells (DCs), macrophages, and natural killer cells. In vitro studies have shown that S1PR3 is essential for S1P-induced maturation, migration, and endocytosis of DCs [69]. Additionally, S1PR3 in DCs plays a key role in recruiting NKT cells and neutrophils to damaged kidneys following IRI. Notably, DCs lacking S1PR3 induce a Th2-like response in NKT cells—specifically prompting them to produce higher levels of IL-4—which protects the kidneys against IRI [69, 93].

S1PR3 is a pro-inflammatory receptor. Hyperglycemia inhibits M2 macrophage polarization and promotes M1 polarization via the S1P/S1PR3 signaling pathway, worsening hepatic IRI. However, pretreatment of diabetic mice with CAY10444 (an S1PR3 antagonist) significantly reduces hepatic IRI [94].

S1PR2 and S1PR3 promote migration of bone marrow-derived monocytes/macrophages by activating the Gi/o/PI3K/Rac1 signaling pathway. Administration of JTE-013 (S1PR2 antagonist) or CAY-10444 (S1PR3 antagonist) to BDL-treated mice significantly reduces recruitment of these cells to fibrotic livers [4, 26]. Yang et al. found that S1PR2/3 activates the Gi/PI3K/JNK signaling pathway, mediating S1P-induced M1 polarization of bone marrow-derived monocytes/macrophages [95]. However, Bryan et al. showed that FTY720 reduces macrophage phagocytic capacity and reactive oxygen species production via S1PR3, thereby reactivating Cryptococcus infection in granulomas [96]. Therefore, S1PR3 exerts dual roles in macrophages: promoting M1 polarization to trigger inflammation while also enhancing phagocytic and killing activities to inhibit bacterial diseases. **Figure 2** provides a schematic overview of these roles.

6 S1PR3 SIGNALING IN NEURAL FUNCTION

Astrocytes are one of the most common cell types in the brain and are critical for maintaining the brain's microenvironment [97]. In vitro studies have demonstrated abundant S1PR3 expression in astrocytes, which increases further during inflammation. S1PR3-G12/13 activates RhoA, leading to increased production of inflammation-related genes [98]. CAY10444, a specific S1PR3 antagonist, has shown protective effects in models of cerebral infarction, spinal cord injury, and intracerebral hemorrhage [99-101].

In vitro experiments show that S1P/S1PR3/Ras/pERK signaling promotes pericyte proliferation, contributing to scar formation after spinal cord injury [99]. Dong et al. found that FTY720P protects astrocytes from oxygen-glucose deprivation-induced injury and inflammation by inhibiting S1PR3-dependent TLR2/4-PI3K-NFκB signaling [101]. Additionally, S1PR3 inhibition exerts neuroprotective effects via the S1P/CCL2/p-p38 MAPK pathway [100]. S1PR3 antagonists may represent a potential therapeutic approach for nervous system injuries.

ED is a hallmark of ARDS [99]. The endothelium is a monolayer of cells that controls the permeability of fluids, proteins and blood cells [86]. In ARDS, endothelial barrier permeability increases as monolayer integrity is disrupted, allowing fluid to enter interstitial tissue, further decreasing gas exchange and inducing hypoxia [102, 103]. Endothelial cell-cell adhesions, including adherens junctions (AJs), tight junctions, and gap junctions, regulate barrier permeability [104]. Among these, AJs have more significant microvascular effects, and expres-

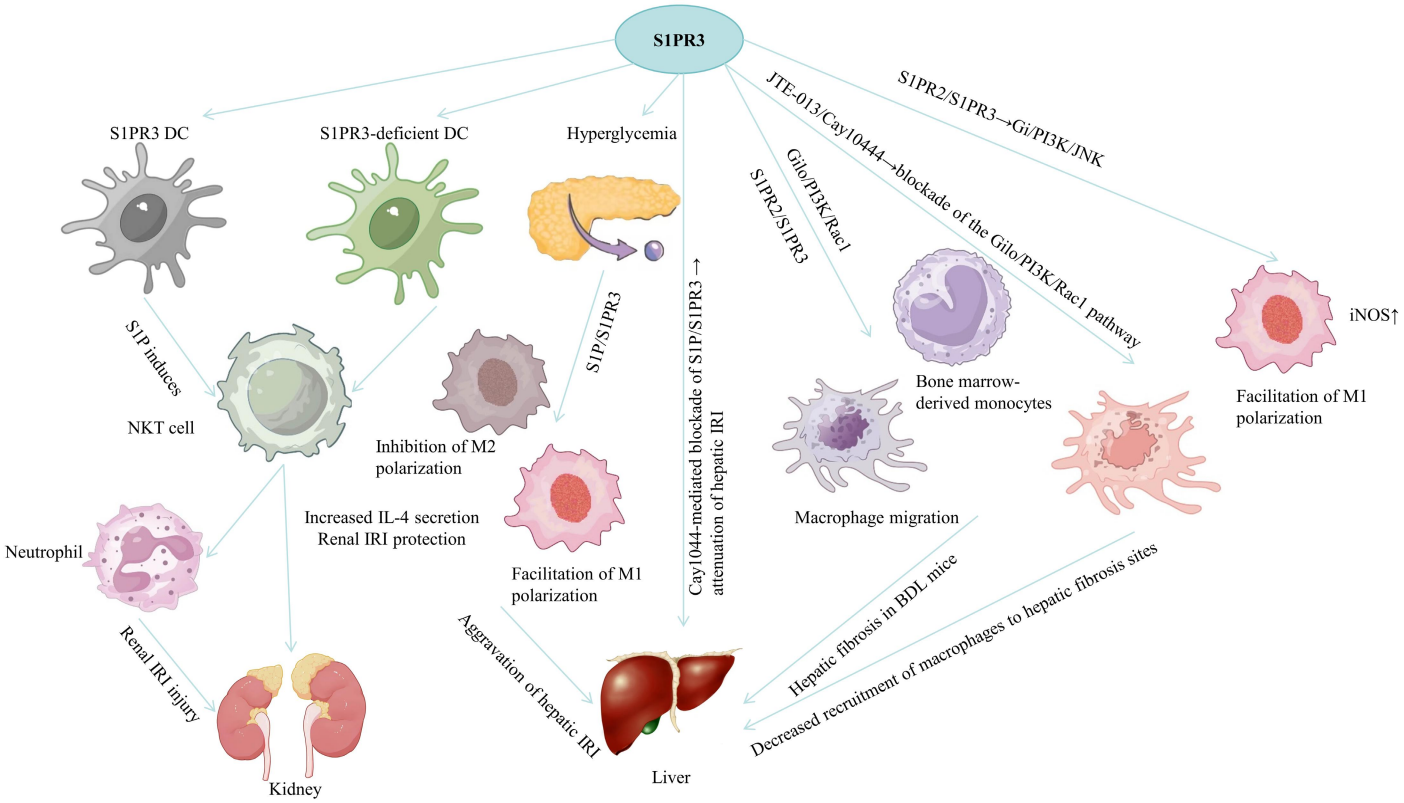

Figure 2. Multifaceted roles of S1PR3 in immune cell regulation and tissue injury responses. S1PR3 orchestrates immune cell functions, including those of dendritic cells (DCs) and macrophages, to mediate renal ischemia-reperfusion injury (IRI), hepatic injury/fibrosis, and anti-infection responses. This figure outlines the multifaceted roles of S1PR3. In renal IRI, S1PR3-expressing DCs (S1PR3 DCs) trigger natural killer T (NKT) cell activation and interleukin-4 (IL-4) secretion, whereas S1PR3-deficient DCs inhibit M2 macrophage polarization, together shaping renal injury outcomes; in hepatic IRI and fibrosis, S1PR3 signals through Gi/o/PI3K/Rac1 or Gi/PI3K/JNK pathways to drive M1 macrophage polarization (marked by elevated inducible nitric oxide synthase (iNOS) expression) and macrophage migration, while S1PR3 blockade with CAY10444 alleviates hepatic IRI and reduces macrophage recruitment to fibrotic lesions; furthermore, hyperglycemia and S1P/S1PR3 signaling amplify M1 polarization to worsen hepatic IRI, and S1PR3 promotes the migration of bone marrow-derived monocytes. Created with Microsoft PowerPoint.

sion of vascular endothelial cadherin (VE-cadherin) at endothelial AJs is essential for endothelial barrier function [48]. AJ structure involves interactions among VE-cadherin, β-catenin, and the actin cytoskeleton [105]. In this study, we found that in LPS-induced ARDS mouse models and LPS-stimulated cell models, S1PR3 inhibition significantly restored expression of VE-cadherin and β-catenin, as shown by Western blotting and immunofluorescence [106]. Additionally, rhodamine phalloidin staining of the cytoskeleton revealed that S1PR3 inhibition significantly reduced LPS-induced cytoskeletal disruption. S1PR3 mediates VE-cadherin mRNA levels in two types of endothelial cells. In a murine ventilator-induced lung injury model, S1PR3 induced endothelial barrier loss through ADAM10-mediated VE-cadherin cleavage [107].

To date, more than 600 clinical trials have targeted Rho signaling [108-110]. In contrast, no clinical trial targeting S1PR3 has ever been conducted. Targeting S1PR3 in clinical trials may be beneficial, as S1PR3 is not only an upstream regulator of Rho but also a mediator of other pathways, such as VE-cadherin cleavage and NFκB-mediated inflammation. Moreover, most existing S1P-modulated drugs targeting S1PR1 agonistically induce downregulation of S1PR1, leading to suboptimal clinical effects and long-term pulmonary function decompensation due to immunosuppression [111]. In contrast, inhibiting S1PR3—a negative regulator of vascular barrier function upregulated during mechanical stress—does not affect S1PR1 expression, ensuring specific therapeutic effects during ventilator-induced lung injury with few side effects under normal lung conditions. However, S1PR3 is also expressed in the cardiovascular system, kidney, and spleen [112, 113]. S1PR3 inhibition could also lead to hypertension, bradycardia, macular edema, reduced pulmonary function, hepatic adverse effects, and neoplasms [114]. Inhalation delivery of S1PR3 inhibitors via aerosol or nanoparticles, which facilitates direct delivery of drugs deep into the airways, may mitigate these effects. Notably, TY-52156, although a selective S1PR3 inhibitor, may carry significant off-target effects, including inhibiting other S1P receptors with relatively lower affinity [115].

S1P, a metabolic mediator of sphingolipid catabolism predominantly formed through SphK1 catalysis, mediates inflammation in sepsis by binding to S1PR3 expressed in macrophages. We demonstrated that the SphK1/S1PR3 axis is upregulated in LPS-induced macrophages and septic mouse lungs, cascading activation of pro-glycolytic signaling molecules such as HIF-1α, HK2, and PFKFB3. Targeted inhibition of SphK1 by PF-543 effectively abrogated the upregulated SphK1/S1PR3 axis in vitro and in vivo. Additionally, PF-543 significantly suppressed sepsis-related inflammation and multi-organ injury in vivo. Furthermore, PF-543 not only blunted key glycolytic enzymes (HIF-1α, HK2, and PFKFB3) in LPS-treated macrophages but also inhibited HK2 and PFKFB3 in septic mice. Silencing or inhibiting SphK1 tempered pro-inflammatory M1 macrophages while boosting anti-inflammatory M2 macrophages. Intriguingly, S1PR3 knockdown efficiently dampened glycolysis-associated markers, reversed LPS-modulated M1/M2 polarization, and attenuated NF-κB p65 activation. In conclusion, this study provides the first evidence that PF-543 orchestrates proportional imbalance of macrophage polarization and the Warburg effect in a SphK1/S1PR3-dependent manner during sepsis, mitigating both hyperinflammation and multi-organ failure, adding a novel puzzle piece to pharmacologically exploitable therapy for sepsis [116].

7 S1PR3-MEDIATED SIGNAL CROSSTALK

Sphingosine-1-phosphate receptors are members of the GPCR family, also known as seven-transmembrane receptors. The peptide chains that traverse the membrane seven times form extracellular and intracellular loops, receiving extracellular signals and initiating intracellular signaling. The intracellular domain interacts with heterotrimeric G proteins, composed of α, β, and γ subunits. Upon ligand activation, the receptor binds G proteins, causing dissociation of the α subunit from the βγ subunits, which then activate downstream effector molecules and regulate biological effects [117].

S1PRs are ubiquitously expressed but exhibit tissue-specific patterns. S1PR1 and S1PR2 are expressed in most tissues: S1PR1 localizes to the plasma membrane, vesicles, cytoplasm, nucleus, and perinuclear regions, while S1PR2 is found in the plasma membrane and cytoplasm. S1PR3 is highly expressed in heart, lung, kidney, and spleen, and is located in the plasma membrane; S1PR4 is mainly expressed in blood cells and lymphoid tissues; S1PR5 is expressed in brain, skin, and natural killer cells [118].

S1PR1 is the most widely expressed S1P receptor in the brain and couples only with Gi/o [83-85]. S1PR2 can couple with Gi/o, G12/13, and Gs, exhibiting highest binding affinity for G12/13 [103, 104, 117-120]. S1PR3 is known to couple with Gi/o, Gq, and G12/13; however, due to its higher affinity for Gq, it can induce intracellular calcium accumulation and PKC activation [88-90]. S1PR4 and S1PR5 can couple with Gs, Gq, and G12/13 [121-127].

S1PR3 has relatively high affinity for Gq proteins. When it forms a heterodimer with CXCR1, ligand binding still prioritizes Gq pathway activation, driving extracellular calcium influx and simultaneously switching on PKC. Once the calcium signal is activated, it further triggers activation of Rho family small GTPases, which then activate downstream Rho-associated protein kinases, regulating cytoskeletal assembly and disassembly by phosphorylating substrates such as myosin light chains. This process adjusts neutrophil morphology and motility, facilitating their migration across the vascular wall to infection sites. Interestingly, this regulatory effect is only significantly evident in neutrophils from pneumonia patients and is induced by S1P. CXCR1/2 G-protein signaling is tightly regulated and quickly desensitized to prevent constitutive signaling through mechanisms including phosphorylation, β-arrestin1/2 recruitment, AP-2 adaptor protein association, and receptor cross-desensitization.Inflammation is a defense mechanism triggered by infection and tissue damage [128]. The CXCL8-CXCR1/2 axis recruits neutrophils to infection sites and induces neutrophil oxidative burst and granule release to eliminate inflammatory stimuli and increase bacterial clearance, thereby protecting the host from further infection and tissue damage [129-131]. Disruption of this axis severely affects host immune mechanisms against infection and may even be fatal. Impaired neutrophil recruitment often leads to decreased bacterial clearance and reduced survival rates in experimental infectious disease models [128].

Given the multiplicity of CXCR1 and CXCR2 ligands and the more selective inhibition of the CXCR1-related pathway via reparixin, the impact of reparixin could be counterbalanced by redundant mechanisms of neutrophil chemotaxis, resulting in perpetuation of inflammation independently of the initial signal. For example, the Duffy antigen receptor for chemokines (DARC), considered a “silent” chemokine receptor, has recently been shown to regulate bioavailability of several chemokines, including CXCL1, CXCL2, CXCL5, and CXCL8, resulting in DARC-enhanced neutrophil trafficking into tissues [132, 133]. The CXCR2 ligand CXCL5 regulates binding site availability for other ELR+ CXC chemokines released during inflammation through its interaction with erythrocyte DARC. CXCL5 inhibits chemokine scavenging, at least in part, through its homeostatic, high-affinity binding with erythrocyte DARC. Thus, in the absence of CXCL5, DARC scavenges proinflammatory chemokines, contributing to reshaping chemokine gradients for neutrophil influx into, for example, the lung [134]. During severe inflammatory responses, such as in a model of Escherichia coli-induced pneumonia, further expression of CXCL5 by alveolar type 2 cells inhibits DARC chemokine scavenging when production of CXCL1 and CXCL2 dramatically increases, resulting in marked increases in circulating plasma concentrations of these chemokines and adverse conse-

quences for efficient neutrophil accumulation [134, 135]. This example illustrates the complexity and alternative pathways of neutrophil recruitment and regulation independent of overwhelming CXCR1 pathway blockade [134].

Cardiac dysfunction has been associated with elevated circulating chemokine levels in both animals and humans. CXCL8 was among the first chemokines detected in the myocardium in response to IRI, and it has been hypothesized to participate in neutrophil-induced myocardial injury. However, several other chemokines, including CCL2 and CXCL10, have been detected early in animal models of myocardial injury. Cardiac myocytes also express other receptors such as CXCR2, CCR2, and CXCR4 (the receptor for SDF-1/CXCL12). These receptors are constitutively expressed and upregulated following oxidative stress both in vivo and in vitro [136]. In an animal model of IRI, Tarzami et al. found a direct myocardial protective action of CXCR2 during IRI, but the magnitude of this protective effect was smaller than the damaging effect of CXCR2-mediated inflammatory cell recruitment [137]. The authors attributed this to different CXCR2-induced signaling between two different cell types—cardiac myocytes and neutrophils [136]. Additionally, new data highlight the relevance of the CXCL12/CXCR4 axis in myocardial IRI. CXCR4 overexpression in vivo worsened cardiac function in an animal model of IRI, enhancing inflammatory cell recruitment, TNF-α production, and cell death, possibly due to increased CXCL12 production [137, 138]. Furthermore, under stress conditions, paracrine and/or autocrine activation of CXCR4 by its ligand may trigger intracellular signaling pathways that exacerbate contractile dysfunction. CXCR4 activation also modulates β2-adrenergic receptor structure and downstream signaling in ventricular cardiac myocytes and has been shown to be a key mediator of calcium handling in adult rat cardiac myocytes [139]. Thus, independently of reparixin action, myocardial IRI may be triggered and maintained by different chemokine/chemokine-receptor inter-relationships (e.g., the CXCL12/CXCR4 axis), which could explain the lack of significant differences in primary endpoints for myocardial IRI in our study [134]. The biological characteristics and functions of S1P and S1PR3 related to these processes are summarized in **Table 2**.

8 CLINICAL APPLICATIONS OF S1PR3

S1PR3 plays a critical role in regulating inflammatory responses and endothelial barrier function in ARDS. It is highly expressed in lung tissue and endothelial cells and has been widely described as a mediator of various biological responses, but its exact role varies across different cells, tissues, and diseases under distinct conditions [140]. Wang et al. reported that S1PR3 inhibition suppressed NLRP3 inflammasome activation in LPS-stimulated macrophages and in the lungs of septic mice [141]. Bajwa et al. reported that S1PR3-deficient dendritic cells exerted protective effects against IRI-induced acute kidney injury [7]. However, the relationship between S1PR3 and ARDS remains unclear. Our results demonstrated that S1PR3 expression was increased in the lungs of LPS-induced ARDS mice, and S1PR3 inhibition alleviated inflammation and endothelial injury both in vivo and in vitro, thereby reducing LPS-induced ARDS [142].

Mechanistically, S1PR3 modulates key inflammatory signaling cascades, including the NF-κB pathway, to orchestrate the production of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 [142, 143]. The NF-κB pathway is considered the central regulator of inflammation and endothelial function in various diseases, and our previous study revealed the importance of the NF-κB pathway in ARDS [143, 144].

NF-κB has five subunits, with p65 being the key transcriptionally active component. IκBα is a crucial controller of NF-κB p65 activation and is phosphorylated by IKKβ to release active NF-κB p65 into the nucleus, thereby promoting transcription of proinflammatory cytokines and chemokines [145]. In endothelial cells, activated NF-κB contributes to VCAM-1 expression, mediating endothelial inflammation and damage [146]. Previous research has indicated an association between S1PR3 and the NF-κB pathway. Yan et al. reported that the NF-κB pathway participates in S1PR3-mediated human renal cell carcinoma progression [147]. Moreover, previous studies have revealed the NF-κB-dependent proinflammatory role of the S1P/S1PR3 axis in lung adenocarcinoma cells, with S1PR3 activation facilitating tumor cell proliferation [136, 148]. Mitochondria, the main powerhouses of cells, are crucial for regulating inflammatory responses and barrier function in endothelial cells [135]. Mitochondrial oxidative phosphorylation is the primary pathway for ATP production, dependent on mitochondrial membrane potential and respiratory chain complex activity [149]. Consistent with previous studies, we found that LPS suppressed mitochondrial oxidative phosphorylation [150]. S1PR3 inhibition decreased reactive oxygen species levels in lung tissues and mitochondrial ROS levels in endothelial cells while increasing the aggregate-to-monomer ratio. Additionally, ATP production and mitochondrial respiratory chain complex activity were restored after S1PR3 inhibition. The complex V inhibitor oligomycin abolished the protective effect of S1PR3 inhibition on ARDS. Therefore, mitochondrial oxidative phosphorylation may mediate the S1PR3 protective effect in ARDS [142].

9 POTENTIAL SIDE EFFECTS OF S1PR3 MODULATION

Preclinical studies have shown that S1PR1 and S1PR3 may be primary contributors to S1P receptor modulator (S1PRM)-induced cardiovascular adverse events [151]. Due to initial S1PR1 agonism, S1PRM administration triggers a decrease in heart rate and blood pressure, activating G protein-coupled inwardly rectifying potassium channels, followed by myocyte hyperpolarization and transient reduction in excitability.

Table 2. Biological characteristics, functions and disease associations of S1P and S1PR3

Core category	Simplified	Related references
S1PRs basic attributes	(1) S1PRs (Sphingosine-1-phosphate receptors, GPCR family) are seven-transmembrane receptors; (2) Extracellular/intracellular loops: receive and initiate signals; (3) Intracellular part interacts with G proteins (α, β, γ); (4) Ligand activation: α-βγ separation→activate downstream effectors.	[117]
S1PRs tissue distribution & localization	(1) S1PRs: ubiquitous, tissue-specific expression; (2) S1PR1: most tissues, abundant in brain; (3) S1PR2: most tissues; (4) S1PR3: high in heart/lung/kidney/spleen; (5) S1PR4: blood cells/lymphoid tissues; (6) S1PR5: brain/skin/NK cells (natural killer cells).	[118]
S1PRs-G protein coupling specificity	(1) S1PR1: only couples with Gi/o; (2) S1PR2: couples with Gi/o/G12/13/Gs (high G12/13 affinity); (3) S1PR3: couples with Gi/o/Gq/G12/13 (Gq→Ca^{2+}/PKC (protein kinase C) activation); (4) S1PR4/5: couple with Gs/Gq/G12/13.	[86-88, 101-103, 119-127]
S1PR3-CXCR1 heterodimer signaling	(1) S1PR3-CXCR1 (C-X-C motif chemokine receptor 1) heterodimer: ligand→prioritize Gq activation; (2) Gq→Ca^{2+} influx+PKC (protein kinase C) activation; (3) Ca^{2+}→Rho GTPases (Rho guanosine triphosphatases)→Rho-associated kinases (Rho-associated kinases); (4) Kinases→regulate cytoskeleton→adjust neutrophil (neutrophil) function; (5) Promote neutrophil migration to infection sites (pneumonia patients: obvious S1P (sphingosine-1-phosphate) effect).	[128]
CXCR1/2 signaling regulation	(1) CXCR1/2 (C-X-C motif chemokine receptor 1/2) signaling: tightly regulated, quick desensitization; (2) Desensitization mechanisms: phosphorylation, β-arrestin1/2 (β-arrestin1/2), AP-2 (adaptor protein 2), cross-desensitization.	[128]
CXCL8-CXCR1/2 axis in inflammation	(1) Inflammation: defense response to infection/damage; (2) CXCL8 (C-X-C motif chemokine ligand 8)-CXCR1/2: recruit neutrophils, induce oxidative burst/granule release; (3) Effects: eliminate inflammation, clear bacteria, protect host; (4) Disruption: impair immunity, reduce clearance/survival.	[128-132]
Redundant mechanisms of neutrophil chemotaxis	(1) Reparixin (Reparixin) effect counterbalanced by redundant pathways; (2) DARC (Duffy antigen/receptor for chemokines): regulates CXCL1/2/5/8 bioavailability; (3) CXCL5 (C-X-C motif chemokine ligand 5) binds DARC and inhibits chemokine scavenging; (4) No CXCL5: DARC scavenges chemokines and reshapes gradients; (5) E. coli (Escherichia coli) pneumonia: CXCL5 upregulation leads to increased CXCL1/2 and affects neutrophils.	[133-136]
Chemokine receptors in myocardial IRI	(1) Myocardial IRI (myocardial ischemia-reperfusion injury): CXCL8 early detected; CXCR2/CCR2 (C-C motif chemokine receptor 2)/CXCR4 (C-X-C motif chemokine receptor 4) upregulated by oxidative stress; (2) CXCR2: mild protection, but severe inflammatory damage.	[135-138]

Note: S1P, Sphingosine-1-phosphate; S1PR3, Sphingosine-1-phosphate receptor 3; LT-HSCs, Long-term hematopoietic stem cells; TNF-α, Tumour necrosis factor-α; NF-κB, Nuclear factor kappa-B; CXCR1, C-X-C chemokine receptor type 1; AML, Acute myeloid leukemia; S1PR3OE, S1PR3 overexpression; PAR1, Protease-activated receptor 1; SphK1, Sphingosine kinase 1; EC, Endothelial cell; LPS, Lipopolysaccharide; IL-8RA, Interleukin-8 receptor alpha.

Continuous administration leads to S1PR1 downregulation, subsequent shifts in the S1P receptor profile, and increased blood pressure [152].

Furthermore, S1PR3 agonism may play a role in heart rate effects: fingolimod induces bradycardia in wild-type mice, but not in S1PR3 knockout mice [153, 154]. However, the potential involvement of S1PR3 in humans remains unclear. Although extensive efforts have been made to develop new modulators with lower affinity for S1PR3 (e.g., siponimod and ozanimod), minimizing S1PR3 binding does not prevent the cardiac effects observed upon first-dose administration [155-159].

A recent study employed gene clustering functional analysis to identify signaling pathways affected by CAY10444 treatment and S1PR3 knockdown, aiming to explore molecular pathways through which targeting S1PR3 influences WEE1 expression [160]. Although CAY10444 treatment affected the PI3K/Akt, PI3K/Akt/mTOR, and MAPK signaling pathways, the PI3K/Akt pathway was the only common pathway affected by both treatments. Studies have reported that S1PR3 can bind to various Gα proteins, including Gi, Gq, and G12/13, thereby regulating signaling pathways such as adenylyl cyclase/cAMP, Ras/ERK/AC, PI3K/Akt, PLC/Ca^{2+}, and Rho/ROCK [161].

Thus, targeting S1PR3 in oral squamous cell carcinoma (OSCC) may inhibit PI3K/Akt signaling pathway activation. The PI3K/Akt pathway exerts crucial biological functions in tumors [162]. Results confirmed that S1PR3 inhibition decreased Akt phosphorylation levels. Conversely, the Akt ago-

nist SC79 restored S1PR3 inhibition-induced suppression of OSCC cell growth both in vitro and in vivo. Additionally, SC79 partially reversed the downregulation of WEE1 and phosphorylated CDC2 induced by S1PR3 targeting. These findings indicate that S1PR3 knockdown inhibits OSCC cell growth by regulating the Akt/WEE1 signaling pathway.

10 CONCLUSION

S1PR3 is a GPCR and seven-transmembrane receptor that binds to extracellular S1P, activating downstream signaling pathways. S1PR3 plays important roles in controlling normal physiological functions and disease processes across multiple systems, including the cardiovascular, immune, nervous, and hepatic systems. S1PR3 exhibits cell- and tissue-specific effects, contributing to both maintenance of normal tissue homeostasis and pathogenesis of diseases such as inflammation, IRI, and atherosclerosis.

This article systematically summarizes the core biological characteristics of S1PR3, its mechanisms of action in various systems, and its prospects for clinical application. It also clarifies the dual roles and regulatory rules of S1PR3 in inflammation, IRI, and atherosclerosis. Notably, conflicting conclusions exist in current research, with the most typical being the controversy over S1PR3's role in myocardial IRI. Some studies have confirmed that activating S1PR3 inhibits cardiomyocyte apoptosis and reduces infarct size via the Gi-Akt-NO pathway; however, others have found that it exacerbates cell damage via the G12/13-RhoA pathway. The core of this contradiction lies in the failure to fully consider the context-dependent activation characteristics of the S1PR3 signaling pathway.

Based on the mechanistic analysis in this article, this controversy can be explained from three aspects. First, differences in injury stage: in the early stage of ischemia (0~2 h after reperfusion), cardiomyocytes secrete low-concentration S1P (approximately 500 nM) in response to stress. At this time, S1PR3 preferentially couples with highly expressed Gi proteins to initiate the PI3K/Akt protective pathway. In the late stage of reperfusion (>6 h), inflammatory factors induce S1P concentrations to rise above 2 μM and simultaneously upregulate G12/13 protein expression, shifting the pathway toward a pro-injury direction. Differences in intervention timing among different studies directly lead to divergent results. Second, cellular heterogeneity: basal Gi protein expression levels in cardiomyocytes are significantly higher than those in vascular smooth muscle cells, and they retain partial Gi coupling activity even in pathological high-concentration S1P environments. However, some studies have failed to distinguish signal differences between cardiomyocytes and interstitial cells, potentially confounding overall injury effects with cell-specific roles. Third, differences in regulatory methods: non-specific agonists (such as S1P itself) are likely to trigger pathway switching due to concentration fluctuations, whereas specific agonists targeting the S1PR3-Gi coupling domain can stably exert protective effects. This also explains why different intervention strategies yield opposite conclusions.

Currently, several deficiencies remain in S1PR3-related research. Besides the PI3K/Akt, PLC/Ca^{2+}, and RhoA/ROCK pathways, crosstalk mechanisms between S1PR3 and other signaling pathways have not been fully elucidated. The specific molecular switches (e.g., specific phosphorylation sites and regulatory proteins) governing S1PR3 signaling pathway conversion under different disease states still need to be identified. Targeted delivery systems and side effect control strategies for S1PR3-specific modulators require optimization. In the future, combining technologies such as single-cell sequencing and proteomics to analyze context-dependent activation mechanisms of the S1PR3 signaling pathway and developing cell-specific S1PR3-modulating drugs will provide new directions for precision treatment of related diseases.

DECLARATIONS

Author contributions

Yichen He, Xiangyi Zhang, and Qin Zhang contributed to the study design and manuscript drafting; Weiqi Lin, Yan Zhang, Haiyi Qian, and Wen Ke were responsible for data acquisition and statistical analysis; Cuifeng Zhang and Qun Chen provided critical insights during data interpretation, participated in constructive discussions, and revised the manuscript for intellectual content.

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Data availability

All source data for this work (or generated in this study) are available upon reasonable request.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Not applicable.

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Perioperative Precision Medicine

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REVIEW ARTICLE

Bacterial infection and sepsis-associated inflammation: Mechanistic insights and emerging clinical perspectives

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Abstract

Sepsis is typically initiated by bacterial infection and the associated immune dysregulation displays a complex, dynamic biphasic immune response characterized by hyperinflammation followed by immunosuppression. In the initial period, pathogens activate the innate immune system through receptors such as Toll-like receptors (TLRs) and NOD-like receptors (NLRs), resulting in the massive release of cytokines, including IL-1β, IL-6, and IFN-γ, thereby initiating a "cytokine storm" that leads to vascular endothelial damage, complement activation, and the induction of abnormal cellular aggregation. Sustained activation of inflammatory signals initiates inflammasome-mediated cell death, including pyroptosis-apoptosis-necroptosis (PANoptosis), leading to systemic inflammation and organ dysfunction. Later, the host develops an immunosuppressive phase characterized by increased anti-inflammatory mediators (e.g., IL-10 and TGF-β), exhaustion of B, T, and NK cells, and immune checkpoint-mediated immune paralysis. The stepwise progression of these two stages suggests that sepsis is not simply a consequence of uncontrolled inflammation but rather reflects immune system reprogramming. A better understanding of the molecular basis of inflammation and immunosuppression, particularly the crosstalk among cell death pathways, metabolic reprogramming, and immune regulation, may facilitate timely precision interventions and the restoration of host immune homeostasis in sepsis.

Keywords: Sepsis, Bacterial infection, Cytokine storm, Immune dysregulation, Inflammatory cell death, Metabolic reprogramming, Immune checkpoints, Precision immunotherapy

1 INTRODUCTION

Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection, and its pathogenesis is generally considered a highly complex process involving pathogen invasion and host immune dysregulation [1]. As one of the most common pathological conditions in intensive care practice, this life-threatening condition is a major cause of infection-related death and is characterized by remarkable heterogeneity and clinical complexity driven by immune and metabolic dysfunction. Technological advances in multi-omics have now shown that sepsis is fundamentally a multidimensional disease mediated by interconnected immune, metabolic, and inflammatory networks and cannot be fully explained by traditional inflammatory paradigms [2].

Bacterial infections are the most common cause of sepsis. Pathogens stimulate the host's innate immune responses via

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various pattern recognition receptors (PRRs), including Toll-like receptors (TLRs) and NOD-like receptors (NLRs), resulting in a cascade of cytokine production and activation of inflammatory signaling. It is this transition from beneficial inflammation to the detrimental cytokine storm that underpins the high mortality of sepsis. Epigenetic regulators, such as peptidyl arginine deiminase 4 (PADI4), and metabolic reprogramming, such as enhanced glycolysis, contribute to inflammasome activation, immune cell exhaustion, and macrophage polarization, thereby reshaping multilayered immune regulatory networks [3]. Pathogens have varying strategies to evade immune responses. For instance, Neisseria meningitidis sustains bacteremia by pilus-dependent adherence to the endothelium lining blood vessels, Group B Streptococcus can compromise the integrity of the neonatal blood-brain barrier leading to neuroinflammation, and Klebsiella pneumoniae uses a coordinated network of virulence factors that co-opt antibiotic resistance mechanisms to facilitate dissemination throughout the body [4-6]. These differences demonstrate that bacterial infections are multidimensional in the pathological development of sepsis.

The inflammatory response is also influenced by host factors that determine both the range and severity of the response. The high incidence of sepsis in the elderly population is biologically associated with increased inflammation via the immunometabolic axis related to aging, metabolic disorders, and gut microbiota dysbiosis. The biphasic immune response underpins the pathophysiology of sepsis: an early phase dominated by hyperactivated innate immune responses and cytokine storm, followed by a later phase of profound immunosuppression and T cell exhaustion—resulting in dynamic dysregulation characterized by "hyperinflammation and immune paralysis". Transcriptomic profiling has enabled the identification of sepsis molecular subtypes, including Sepsis Response Signature 1 (SRS1; immunosuppressive) and SRS2 (proinflammatory/activated), thereby providing a conceptual framework for patient stratification and potential personalized therapeutic strategies [7]. Meanwhile, metabolic reprogramming has been identified as an important driver of immune hyporesponsiveness. Excessive glycolysis and impeded oxidative phosphorylation not only weaken the efficacy of immune cells but also aggravate metabolic dysregulation and organ damage, thus magnifying immunosuppression.

Furthermore, comprehensive systems-level modeling of these pathophysiological networks is advancing sepsis research beyond a single inflammatory paradigm. Many signal transduction pathways, such as NF-κB, MAPK, and JAK/STAT, converge in different tissues giving rise to complex inflammatory networks. Conversely, immune suppression and tissue repair in the late phase of inflammation are induced by negative feedback loops such as autophagy, the nuclear factor erythroid 2-related factor 2 (Nrf2)-mediated antioxidant pathway, and thymic stromal lymphopoietin (TSLP), which may reflect this shifting balance between host defense and restoration of homeostasis [8]. The diagnostic paradigm of sepsis is evolving across evidence, clinical trials, and definitions. Clinical research is even changing the model of diagnosis for sepsis, shifting from traditional symptom-based scoring systems toward molecular multi-omics–based diagnostic frameworks. The simultaneous assessment of biomarkers and metabolic markers has substantially improved the sensitivity of early diagnosis, while host genetic polymorphisms have offered a genetic basis for individual susceptibility. Immunophenotypes and metabolism-based precision stratification approaches are paving the way for the concept of personalized immune reconstruction as a potentially curative therapeutic strategy.

Sepsis is not a random consequence of repeated immune activation but rather reflects systemic breakdown under persistent infectious challenge. Bacterial infection disrupts the balance between immune and metabolic processes, driving metabolic reprogramming of immune cells and subsequent immune dysfunction. For example, sepsis can induce persistent activation of $CD4^+$ T cells accompanied by non-specific IL-17 production, a process associated with mitochondrial dysfunction and enhanced glycolysis that may persist long after the initial infection and impair host responses to secondary challenges [9]. These alterations ultimately drive the host into a state of systemic instability that extends beyond localized inflammation and progresses toward systemic physiological reorganization.

Advances in multi-omics technologies and systems biology have further reshaped the understanding of sepsis pathogenesis. Evidence indicates widespread alterations in immune and metabolic signaling pathways in septic patients, with several pathways correlating with immune cell abundance and patient survival [10]. These system-level perturbations highlight that sepsis is not merely an exaggerated inflammatory response but a complex immune-metabolic dysregulation involving multilayered regulatory networks. Because this dysregulation originates from host responses to invading pathogens, understanding the molecular mechanisms of bacterial infection is essential for elucidating how localized infection progresses to systemic sepsis.

2 MECHANISMS OF BACTERIAL INFECTION AND THE PROGRESSION OF SEPSIS

2.1 Bacterial invasion and host response

The initiation of sepsis is a classical example of pathogenic attack and host response imbalance. In this process, bacterial invasion activates PRR-mediated inflammatory cascades. This host defense immune response directed against pathogen elimination is exaggerated and becomes self-amplifying, particu-

larly within cytokine signaling networks in a process known as a "cytokine storm". Such an overwhelming response is thought to stimulate the release of numerous cytokines, leading to microcirculatory dysfunction and eventually multiple organ failure [11]. In recent years, system-wide proteome studies have also identified that dysregulated activation of the complement and coagulation cascades, together with metabolic reprogramming, constitutes key molecular networks responsible for septic liver injury. These molecular imbalances are functionally related and ultimately contribute to immune and organ dysregulation [12].

The delicate structure and precise regulation of immune responses have become more clearly delineated in recent years. Loss of the epigenetic regulators PADI2 or PADI4 suppresses NLRP3 inflammasome activation and promotes M2 macrophage polarization, thereby reducing tissue inflammatory injury [3]. This discovery not only demonstrates the importance of epigenetic changes in the course and outcome of sepsis but also indicates that modulating immune cell fate may provide a new therapeutic avenue for this disease. Clinically, the Liverpool quick Sequential Organ Failure Assessment (LqSOFA) instrument correlates with downstream parameters of immune and circulatory dysregulation by providing a scoring system that represents the degree of organ dysfunction [13]. Various pathogens further aggravate immune dysregulation through distinct mechanisms. N. meningitidis sustains bacteremia by dynamic retraction of type IV pili, which themselves drive a positive feedback loop between inflammation and the coagulation cascade [4]. In contrast, Group B Streptococcus itself can bypass the neonatal blood-brain barrier and cause neuroinflammation and white matter damage. Importantly, this pathological course is independent of hypoxic pathways, emphasizing the infection-mediated neuropathological phenotype that is characteristic of bacterial sepsis [5]. In fetal sepsis, organisms traverse the placenta and are distributed to a number of organs, indicating a twofold mechanism of "direct pathogen invasion combined with dysregulatory host immune factors" [14].

Increasing attention has been paid to the role of gut-derived pathogens in sepsis. Klebsiella pneumoniae can cause systemic infection through translocation from the intestinal compartment, facilitated by pks^+ virulence traits and antibiotic resistance that undermine host barriers and promote organ colonization [6]. On the other hand, age-related remodeling of the gut microbiota substantially promotes pathogen immune evasion, leading to exaggerated inflammatory responses and organ injury, which may provide a biological interpretation for the increased susceptibility of elderly individuals [15]. Metabolic conditions also profoundly influence the course of inflammation. Immune cells can be activated under hyperglycemic conditions, promoting glycolytic pathways and aggravating inflammation and lactic acidosis; moderate glucose restriction contributes to metabolic homeostasis via gluconeogenesis, thereby mitigating inflammation and organ injury [16]. Furthermore, some pathogen surface proteins, such as GPAC, can rapidly trigger neutrophils to secrete vasoactive products and TNF-α, thereby escalating the pathological inflammatory response [17]. Other environmental conditions, such as prolonged alcohol exposure, also enhance systemic and gut inflammatory responses by compromising intestinal barrier function with concomitant increased release of Th1/Th17 cytokines [18].

These endogenous host pathways are also important mechanisms for immunological dysregulation. Skeletal muscle Myeloid differentiation primary response 88 (Myd88) signaling regulates sex-specific cytokine release and neutrophil recruitment, with consequences for sepsis survival [19]. Importantly, recent investigations have indicated that modulating the host in addition to targeting pathogens may provide new treatments for sepsis. For example, CYP1A1 has been identified as a negative regulator of host anti-infective responses. Its small-molecule inhibitors may modulate macrophage phagocytic function and exert therapeutic effects against multidrug-resistant bacterial infection [20]. This finding suggests a potential therapeutic strategy for future interventions: reshaping immune and metabolic networks, interrupting the vicious circle of inflammation and organ damage, and offering novel ways for precision medicine interventions in sepsis. While bacterial invasion and systemic dissemination define the pathological onset of sepsis, the pivotal transition occurs at the molecular interface where pathogen-associated signals are sensed and translated into immune activation. This shift from invasion to recognition marks the progression from localized infection to systemic inflammatory dysregulation. Consistently, the mature regulatory dendritic cell program is induced within ~24 h after septic challenge through TNFRSF–NF-κB and IFNGR2–JAK–STAT3 signaling. Analysis of publicly available single-cell datasets from patients with COVID-19 further confirmed the presence of mregDC populations in human sepsis-associated immune responses, underscoring the importance of pathogen sensing and downstream immune activation in shaping septic inflammation [21].

2.2 Pathogen recognition and immune activation

Pathogen recognition and subsequent immune activation are central in the initiation and progression of sepsis. Molecular sensors in both host and microbial compartments, including PRRs and inflammasomes, recognize pathogen-associated invasion signals and initiate early host defense responses. However, this mechanism may be dysregulated and progress into destructive inflammation during sepsis, further leading to organ damage and immune failure.

At the innate immunity level, neutrophils and erythrocytes have a much broader function than was previously believed. The Sema7A-PlexinC1 signaling axis promotes adhesion, neutrophil chemotaxis, and platelet aggregation to enhance the pulmonary inflammatory response; Erythrocytes not only rec-

ognize microbial DNA via TLR9 but also trigger macrophage activation and inflammatory cytokine release, revealing a previously unrecognized role of erythrocytes in pathogen detection [22, 23]. Meanwhile, the NLRP6 inflammasome induces overwhelming inflammation via IL-18 and impairs T cell survival, facilitating host immune dysfunction and collapse [24]. The simultaneous release of bacterial outer membrane vesicles and ATP can locally suppress neutrophil function, mediate degranulation, and disrupt systemic inflammatory balance, generating a "double-edged sword" mechanism between pathogen and host responses [25, 26].

Pathogen-derived immune-evasion strategies further aggravate host immune dysregulation. Streptococcus pneumoniae BgaA promotes adhesion and resistance to bacterial killing, thereby activating coagulation pathways and aggravating tissue damage, which further intensifies inflammatory pathology; Klebsiella pneumoniae is antibiotic resistant, and thus successfully evades immune clearance and perpetuates the inflammation-damage cycle; Group B Streptococcus enhances biofilm formation and invasion with the aid of its LytSR system by interfering with host immune recognition [27-29]. Together, these pathogen-specific mechanisms contribute to immunological dysregulation during sepsis.

At the level of the immunological network, sepsis is characterized by reduced adaptive immune activation together with excessive innate immune activation. Consistent with the therapeutic relevance of infection-driven immune dysregulation, IOX1 has been shown to regulate endotoxemia and bacterial sepsis caused by Escherichia coli and carbapenem-resistant Acinetobacter baumannii, while suppressing multidrug-resistant bacterial growth through inhibition of DNA gyrase [30]. In parallel, postoperative sepsis further exemplifies immune suppression within this dysregulated network, as persistent CTCF binding to the major histocompatibility complex (MHC)-II region prevents the expression of CIITA and human leukocyte antigen (HLA)-II genes, thereby directly impairing antigen-presenting function and worsening immune paralysis [31].

Of note, host and bacterial metabolites are involved in regulating the magnitude of inflammation. Vascular endothelial growth factor (VEGF) enhances the phagocytic and lysosomal functions of endothelial cells through cyclic adenosine monophosphate–transcription factor EB signaling, thereby promoting the elimination of intracellular Streptococcus pyogenes; by contrast, VEGF-depleted vessels show impaired bactericidal activity and support bacterial dissemination [32]. The gut-derived metabolite indole-3-lactic acid targets PFKFB2 to suppress glycolytic flux and reduce cytokine storm severity and organ injury [33]. Concurrently, DJ-1 deletion induces autophagolysosome formation, and enhances bacterial clearance and immune response [34]. These findings demonstrate the importance of "immune-metabolic coupling" in controlling inflammation during sepsis. In addition, some endogenous molecules show promise in protecting against inflammation. Rhamnose inhibits TLR4/ROS-mediated caspase-1 activation and diminishes IL-1β/IL-18 release, attenuating pyroptosis and oxidative injury caused by resistant bacteria; DLL1, as a ligand for monocytes, specifically boosts inflammatory signaling in infection by enabling the immune response to discriminate infectious from non-infectious inflammation [35, 36]. Although complement is acutely activated in early sepsis, its function becomes decoupled from disease progression and patient outcome, and it may play a role more as an early pathologic trigger than as a driving force in later stages [37].

These findings indicate that pathogen recognition and immune activation in sepsis constitute a complex and dynamically dysregulated network involving classical PRR and inflammasome signaling, pathogen immune-evasion strategies, and host immunometabolic crosstalk. This framework not only deepens our understanding of sepsis immune pathology but also highlights potential strategies for precision diagnosis and therapy by restoring the balance between host defense and inflammatory injury.

Within this network, inflammatory signaling pathways interact across multiple molecular signals to reinforce the inflammatory response, driving the transition from localized immune responses to systemic inflammatory dysregulation. Cytokines play pivotal roles in this process, exerting dual effects in sepsis: excessive activation can trigger a "cytokine storm", whereas persistent dysregulation may lead to immunosuppression [38]. The extensive cross-activation among these pathways forms a highly interconnected inflammatory regulatory network (**Table 1**) and ultimately converges on the molecular drivers of inflammatory storms. Although pathogen recognition initially serves as a protective response, sustained amplification of these signaling networks promotes the release of cytotoxic mediators and bacterial toxins, which directly damage cellular structures and organelles. This transition from immune activation to toxin-mediated cellular injury represents a critical step in the progression of sepsis pathogenesis.

2.3 Bacterial toxins and host cell damage

Bacterial toxins and host response dysregulation induce cellular injury and organ failure. Several signaling pathways and organelle injury processes have been implicated in sepsis, indicating that they are not only part of the pathological mechanism but also potential therapeutic targets.

In skeletal muscle, SPSB1 worsens sepsis-induced muscle atrophy and regeneration failure through inactivation of the TβRI–Akt–myogenin signaling pathway and suppression of protein synthesis [69]. Intestinal ACE2 can modulate the gut microbiota and the metabolite 5-MTP, activate the PI3K–AKT–WEE1 pathway, and support barrier function, thereby mitigating sepsis and multi-organ damage [70]. In the myocardium, actin/

Table 1. Roles of inflammatory cytokine networks and signaling pathways in cytokine storms

Inflammatory/ regulatory factors	Upstream triggering mechanisms	Major signaling pathways	Downstream effects	Clinical significance	References
miR-30d-5p	TNF-α stimulates polymorphonuclear neutrophils to release exosomes	Upregulates NLRP3 inflammasome via the NF-κB signaling pathway	Induces M1 polarization and pyroptosis in macrophages	Potential novel therapeutic target for sepsis-associated ALI	[39]
IL-1β	Polymicrobial sepsis induced by cecal ligation and puncture (CLP)	Activates NF-κB and autophagy via the NLRP3/IL-1β pathway	Leads to cardiomyocyte atrophy and impaired systolic and diastolic function	Inhibition of NLRP3/IL-1β may prevent sepsis-induced cardiomyopathy	[40]
IL-6	Sepsis induced by CLP	Cortical TGF-β1/Smad3 and medullary IL-6/STAT3 signaling pathways	Mitigates renal epithelial-mesenchymal transition, apoptosis, and inflammation	PRMT1 may serve as a novel therapeutic target for sepsis-induced kidney injury	[41]
IL-8	Sepsis-induced neuroinflammation	Cytokine–receptor interactions and IL-17 signaling pathway	Leads to delirium, coma, and other neurological dysfunctions	IL-8 may serve as a diagnostic marker and therapeutic target	[42]
IL-10	Excessive inflammation induced by LPS or sepsis	IL-10–mediated anti-inflammatory response	Suppresses pro-inflammatory cytokine production and improves survival	$CD169^+$ macrophages may serve as a novel target for anti-inflammatory therapy	[43]
IFN-γ	Macrophages stimulated with LPS combined with IFN-γ	Inhibits PINK1-mediated mitophagy via STAT1-dependent pathway	Promotes macrophage activation and bacterial clearance	Mitophagy inhibition may improve sepsis outcomes	[44]
MCP-1	Polymorphism of MCP-1 gene rs1024611 G allele	Upregulation of MCP-1 expression promotes inflammatory responses	Increased pro-inflammatory cytokines; susceptibility to sepsis in diabetes	MCP-1 polymorphism may serve as a risk marker for diabetic sepsis	[45]
HMGB1	Sepsis-induced hyperlactatemia	HMGB1 modifications mediated by GPR81/Hippo/YAP and p300/CBP	Lactylation/acetylation of HMGB1 and its release via exosomes	Targeting lactate/GPR81 signaling may improve sepsis outcomes	[46]
IL-12	Sepsis induced by LPS or CLP	Heat shock protein 40-like protein 1 (HLJ1)/IL-12/IFN-γ axis	Enhanced IFN-γ production by NK cells leads to hyperinflammation and mortality	Targeting HLJ1 may provide therapeutic intervention for IL-12/IFN-γ axis–mediated sepsis	[47]
IL-18, NLRP3	Sepsis-associated acute kidney injury (SA-AKI)	Activation of the NLRP3 inflammasome pathway	Promotes inflammatory responses, leading to renal dysfunction	Potential diagnostic biomarker for SA-AKI	[48]
IL-17	Sepsis-induced pancreatic injury via CLP	RhoA/ROCK and STAT3/RORγt signaling pathways	Suppresses Th17 differentiation and pancreatic cell apoptosis	Combination of Fasudil with SR1001 may exert synergistic therapeutic effects in sepsis	[49]
TGF-β	Sepsis- or LPS-induced inflammatory response	Regulates glycolysis via the TGF-β/mTOR/c-MYC signaling pathway	Enhances glycolysis while suppressing pro-inflammatory cytokines, promoting coagulation	TGF-β may serve as a therapeutic target in sepsis	[50]
IL-4, Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF)	Activation of B and T cell Nociceptin/Orphanin FQ receptor (NOP) system induced by LPS/PepG stimulation	N/OFQ-NOP autocrine signaling pathway	Inhibits cell migration and reduces GM-CSF release	NOP antagonists may serve as potential therapeutic agents for sepsis	[51]
Aquaporin-3 (AQP3), IL-33	Upregulation of AQP3 expression due to CC genotype	High AQP3 expression is associated with elevated IL-33 levels	Significantly reduced 30-day survival in patients	AQP3 polymorphism may serve as a prognostic marker	[52]
C5a	Excessive complement C5a production in sepsis	Activation of MAPK/AKT kinase pathways via C5aR	Induces apoptosis of adrenal medulla cells and dysregulation of inflammatory proteins	C5a inhibition may protect adrenal function and improve sepsis outcomes	[53]

Plasminogen activator inhibitor-1 (PAI-1)	4G/5G polymorphism of the PAI-1 gene	Elevated PAI-1 levels affect neutrophil activity	Altered neutrophil activation and increased mortality	Modulating PAI-1 may improve sepsis outcomes	[54]
Ang-2, VEGF-A	Sepsis-induced vascular barrier disruption	Dual targeting of Ang-2/VEGF-A	Reduces inflammatory cell migration and cytokine release	A2V bispecific antibody may serve as a novel therapeutic agent for restoring vascular barrier in sepsis	[55]
S100A8/A9	High levels of S100A8/A9 released by PMNs in sepsis	S100A8/A9–Nrf1–mitochondrial complex I–ZBP1 signaling pathway	Induces mitochondrial dysfunction and endothelial cell PANoptosis	S100A8/A9-high neutrophils may serve as prognostic markers and therapeutic targets	[56]
MIP-1α	LPS-induced sepsis model	Regulates the RAAS pathway and nitric oxide levels	Attenuates pulmonary and renal inflammation and tissue injury	Dexamethasone may serve as a potential therapeutic strategy for sepsis	[57]
RANTES	Immune dysregulation triggers a pro-inflammatory milieu	Monocyte-mediated pro-inflammatory cytokine network	Elevated pro-/anti-inflammatory ratio distinguishes sepsis	Serves as a potential diagnostic biomarker to differentiate sepsis from malaria	[58]
IL-22	CLP-induced sepsis and lung injury	IL-22 activates the STAT3 signaling pathway	Inhibits pulmonary epithelial apoptosis and alleviates inflammation	IL-22 may represent a novel therapeutic strategy for sepsis-induced lung injury	[59]
IL-27, IL-27Rα	Neonatal sepsis induces elevated IL-27 expression	IL-27 signaling regulates inflammatory responses	Suppresses excessive inflammation and enhances bacterial clearance	IL-27 antagonism may serve as a novel therapeutic strategy for neonatal sepsis	[60]
IL-35	LPS induces inflammatory activation of endothelial cells	IL-35 activates the STAT1/STAT4 signaling pathway	Inhibits apoptosis and the expression of inflammatory mediators	IL-35 may serve as a potential endothelial-protective strategy in sepsis	[61]
IL-37	Sepsis-induced inflammatory response in lung injury	Mediates apoptosis through the TGF-β/Smad3 signaling pathway	Attenuates pulmonary edema, inflammation, and apoptosis	IL-37 may represent a potential therapeutic target for sepsis-induced lung injury	[62]
IL-38	LPS induces IL-38 expression in macrophages	The IL-38/IL-36R axis regulates macrophage polarization	Inhibits apoptosis and NLRP3 inflammasome activation	IL-38 may serve as a novel immunoregulatory therapeutic target in sepsis	[63]
sTREM-1, sST2	Sepsis-induced activation of immune responses	Inflammatory pathways correlated with SOFA scores	Enhance diagnostic accuracy and prognostic assessment in sepsis	Combined detection may represent a novel diagnostic strategy for sepsis	[64]
IL-23	Sepsis induces excessive inflammatory responses	Activation of IL-23–related inflammatory pathways	Elevated pro-inflammatory levels associated with increased mortality	May serve as an independent biomarker for sepsis diagnosis and prognosis	[65]
AKK, RKH, TLR4	Sepsis reduces intestinal abundance of AKK	RKH directly antagonizes the TLR4 signaling pathway	Suppresses excessive inflammation and mitigates organ injury	AKK or RKH may represent potential therapeutic strategies for sepsis	[66]
Sphingosine kinase 1 (SphK1), Sphingosine-1-phosphate (S1P), S1PR1	Acute ethanol intoxication aggravates sepsis-induced lung injury	Activation of the SphK1/S1P/S1PR1 signaling pathway	Increases vascular permeability, inflammation, and apoptosis	Inhibition of SphK1 may represent a potential therapeutic strategy	[67]
GSDMD	S100A8/A9 activates platelet TLR4 during sepsis	S100A8/A9–TLR4–GSDMD pyroptosis pathway	Triggers platelet pyroptosis, promotes NET formation, and amplifies inflammatory cascades	Inhibition of the S100A8/A9–TLR4 axis may represent a novel therapeutic strategy for sepsis	[68]

myosin degradation by calpain-1 plays a major role in sepsis-induced heart injury. Interventions targeting calcium signaling can mitigate cardiac dysfunction and increase the survival rate [71]. Extracellular histones are also detrimental to the heart, as they cause imbalances in calcium homeostasis and mitochondrial function, resulting in cardiac failure. Inhibition of histones is a novel therapeutic strategy [72].

The impairment of both endothelial and barrier function of vasculature is especially important in the development of sepsis. Sepsis-induced disseminated intravascular coagulation disrupts EXT1/FGFR1-mediated glycocalyx remodeling and enhances barrier damage and pulmonary edema; TGF-β1 derived from platelets induces neutrophil recruitment and promotes extracellular trap formation, leading to thrombosis as well as organ injury [73]. Its absence increases survival, and makes it a candidate therapeutic target [74]. At a later stage after shock, the Angiopoietin-2 (Ang-2)/Tie2 pathway is involved in vascular leakage, and subsequently contributes to remote, indirect Acute Respiratory Distress Syndrome (ARDS).

Inhibition of Ang-2 could restore barrier function and increase survival rate [75]. Additional studies demonstrate that Ang-2 also induces lung injury and increases inflammation by modulating endothelial-neutrophil interactions [76].

Mitochondrial injury and dysfunction are key elements of sepsis-induced organ failure. Mitochondria-related hub genes have been shown to influence energy metabolism and immune microenvironment regulation, which are significantly involved in the pathogenesis and progression of Sepsis-ACM [77]. Histone Deacetylase (HDAC) 3 inhibits mitochondrial quality control by the FOXO1–Rho-associated coiled-coil containing protein kinase (ROCK) 1 axis, driving progression of acute lung injury (ALI); thus, HDAC3 inhibition might represent a promising novel therapeutic opportunity [78]. The mechanosensitive ion channel Piezo1 is triggered during sepsis to induce calcium influx and mitochondrial impairment which in turn disrupts the tight junctions of the intestinal epithelial cells, followed by cellular apoptosis, thereby propagating barrier damage [79].

Immune and reparative functions are also suppressed in the long term. Sepsis may lead to the dysfunction of bone marrow (BM) mesenchymal stem cells (MSCs), which is characterized by mitochondrial depolarization and decreased cytokine expression, thereby disrupting BM homeostasis and long-term immune reconstitution [80]. Recurrent sepsis worsens $CD4^+$ T cell exhaustion, interferes with antiviral immunity, and is consequently associated with long-term mortality, demonstrating the sustained immune consequences of sepsis [81]. Furthermore, artesunate and metabolism-related proteins also show therapeutic potential by regulating hepatic endothelial cell subpopulation proportions, inhibiting inflammatory cytokine release in liver macrophages, and decreasing lymphocyte apoptosis, thereby alleviating liver injury and immune imbalance and supporting their potential clinical application [82]. VDBP is reduced with sepsis and correlated with liver injury and mortality. Its overexpression could protect against oxidative stress and hepatocyte injury, which may have a protective role [83].

Interactions between bacteria-derived molecules and host response mediators constitute central driving forces in the pathogenesis of sepsis. Emerging evidence highlights the critical role of inflammatory cytokine networks in this process. For example, the combined action of tumor necrosis factor with IL-18, IFN-γ, or IL-1β is sufficient to reproduce sepsis-associated tissue injury, indicating that host inflammatory mediators alone can drive systemic pathological alterations [84]. Concurrently, metabolic disturbances in skeletal and cardiac muscle are closely associated with endothelial barrier disruption and immune dysfunction, thereby facilitating the transition from localized inflammatory injury to systemic pathology. Sepsis is therefore characterized by a complex and highly lethal pattern of multi-organ damage, with key pathological hubs including mitochondrial dysfunction, vascular barrier breakdown, and immune exhaustion. These pathological nodes not only reveal the underlying logic of disease progression but also represent potential targets for precision therapeutic interventions. For instance, recombinant angiopoietin-like protein 4 has been shown to significantly reduce vascular leakage, organ failure, and mortality in murine models of lethal sepsis and Neisseria meningitidis infection, further underscoring the critical role of endothelial barrier integrity in disease progression [85]. When the immune system is rapidly mobilized to combat infection, this protective response can escalate into a systemic storm of signaling mediators. Inflammatory cell death and cytokine release mutually reinforce one another, forming a self-sustaining inflammatory loop (**Figure 1**) that drives the progression of inflammation from local dysregulation to systemic inflammatory imbalance. Within this cascade, aberrant activation of immune cells together with uncontrolled release of inflammatory mediators becomes a central force sustaining the inflammatory response, thereby laying the mechanistic foundation for subsequent discussions on immune regulation and inflammatory signaling.

3 IMMUNE SYSTEM REGULATION IN INFLAMMATORY RESPONSES

3.1 Activation of immune cells and release of inflammatory cytokines

In sepsis-induced ALI and multiple organ dysfunction syndrome, the devastating cascade is mainly due to the abnormal activation of immune cells and the dysregulated release of inflammatory mediators, which triggers a harmful inflammatory cascade. Neutrophil-derived exosome miR-30d-5p was found to promote macrophage M1 polarization and pyroptosis, thus representing a critical element that drives lung injury [39].

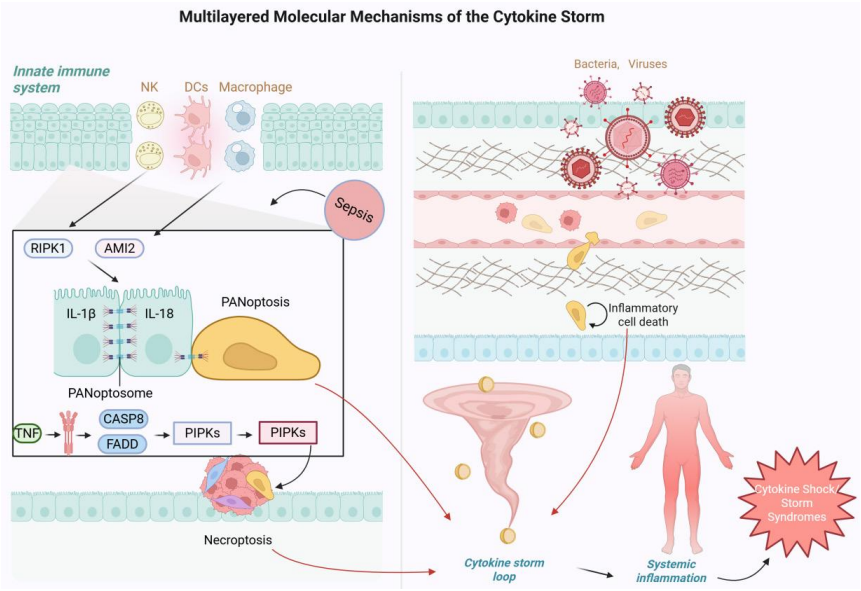

Figure 1. Multi-layered molecular mechanism of inflammatory storm. After pathogen invasion, the innate immune system is quickly activated by NK cells, dendritic cells and macrophages, inducing a vast amount of inflammatory mediators. This results in an inflammatory cascade focused on TNF, IL-1β and IL-18. The AIM2-dependent pathway works together with RIPK1 to promote the formation of the PANoptosis complex, including the assembly of PANoptosome components such as CASP8 and FADD, thus leading to inflammatory cell death and cytokine amplification. A variety of programmed cell death modalities, including pyroptosis and necroptosis, are interwoven to synergistically amplify tissue damage, as illustrated in the left panel of the figure, leading to the formation of the "cytokine storm loop". The right panel further depicts how inflammatory cell death and cytokine release disrupt tissue barriers and propagate inflammation systemically. Such a self-perpetuating inflammatory network can transgress tissue boundaries, producing a systemic inflammatory response and ultimately contributing to multiple organ failure and cytokine shock syndromes.Created by the authors using BioRender.

eCIRP facilitates the generation of aging neutrophils with augmented antigen presentation, leading to Th1 differentiation and IFN-γ–dependent accelerated high-level Neutrophil Extracellular Trap formation (NETosis). This amplifies the inflammation and damages tissues [86]. Furthermore, manganese ions also enhance lipopolysaccharide (LPS)-induced innate immune responses through activation of the cyclic GMP-AMP synthase–stimulator of interferon genes pathway, which leads to severe septic shock [87]. In the nervous system, septic plasma exosomes can induce microglial activation via TLR7/MyD88 signaling and result in neuroinflammation, which is associated with the pathogenesis of sepsis-associated encephalopathy (SAE) [88]. In addition to pro-inflammatory responses, protective physiological countermeasures are present in the body. S-phase kinase-associated protein 2 (Skp2) relieves tissue injury by suppressing ferroptosis in lung epithelial cells; deletion of G protein-coupled receptor 174 (GPR174) enhances regulatory T cell (Treg) function and M2 macrophage polarization, thereby inhibiting excessive inflammation [89, 90]. Furthermore, DPP4 and TXN have been suggested as probable indicators for immune-regulation functions, which can offer new molecular targets for precision therapy [91].

The intersection of metabolism and inflammation is also important. Tripterygium wilfordii glycosides inhibit the Warburg effect via PKM2/high mobility group box 1 (HMGB1), which attenuates inflammation and organ damage, while neutrophil extracellular traps (NETs) promote ferroptosis by Methyltransferase-Like (METTL)3-mediated N6-methyladenosine (m^6A) modification of Glutathione Peroxidase 4 (GPX4), aggravating lung injury [92, 93]. In addition, interactions among inflammatory factors are also important. TNF-α and IFN-γ induce PANoptosis through the JAK/STAT1/IRF1 axis to cause severe cell death and tissue damage. This process is also observed in COVID-19 and sepsis [94]. On the other hand, the lactate-enhanced qSOFA score has shown good predictive value for in-hospital mortality in patients with sepsis; mechanistically, LPS/IFN-γ inhibit PTEN-induced kinase 1 (PINK1)-mediated mitophagy through the STAT1-caspase pathway, thereby promoting mitochondrial reactive oxygen species production, bacterial clearance, and improved survival during sepsis [13, 44].

Immune activation in sepsis occurs through multiple layers of pro-inflammatory amplification, including extracellular exosomal miRNAs, NET formation, and PANoptosis, which collectively drive acute organ injury. However, persistent inflammatory stress progressively reprograms immune regulatory networks, shifting the host response from hyperinflammation toward immune exhaustion and dysregulation. This transition ultimately leads to the immune imbalance that defines the later stages of sepsis.

3.2 Immune system imbalance and hyperreaction

Following this storm of inflammation, there is a rapid transition toward an immunosuppressive state in sepsis patients, with the establishment of a classic "immune imbalance". Reduced expression of HLA-DR on monocytes is a hallmark of this stage and is strongly related to poor prognosis [95]. In terms of molecular mechanisms, p53 stimulates immunosuppression through inhibition of $CD4^+$ T cell proliferation and upregulation of apoptotic pathways, ultimately disrupting T cell homeostasis; the absence of Sulf-1 alters endothelial heparan sulfate modifications, resulting in glycocalyx dysfunction that mediates pulmonary immunosuppression [96, 97]. The emerging role of non-coding RNAs in regulation of

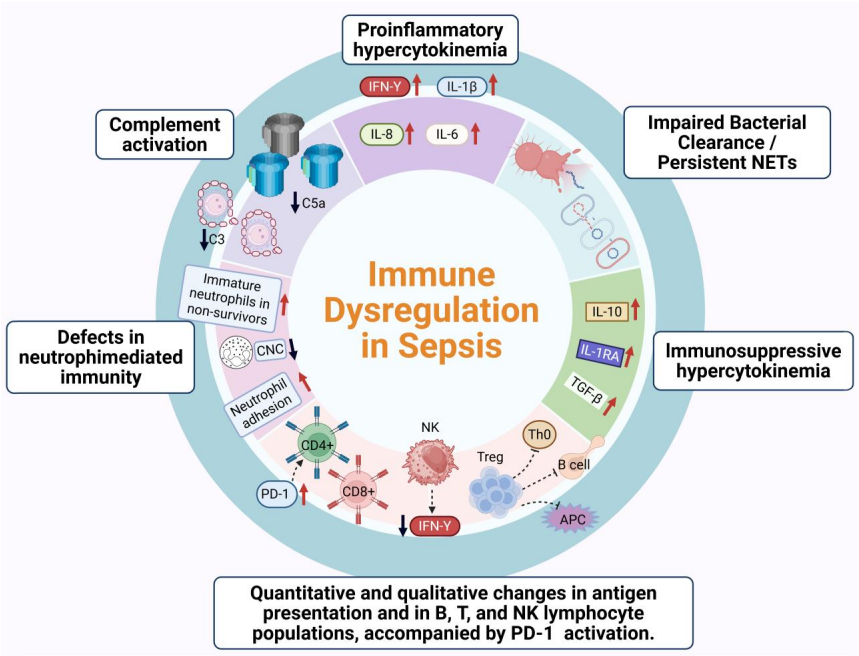

Figure 2. Pathophysiological mechanisms of immune imbalance in sepsis. The immune dysregulation induced by sepsis is a biphasic response in which proinflammatory and immunosuppressive phases succeed each other, as illustrated in the circular schematic showing interconnected immune pathways. At the very beginning, over-activation of the complement system (C3, C5a) and neutrophil dysfunction amplify inflammation to an overwhelming level, as exemplified by a dramatic rise in factors like IL-1β, IL-6 and IFN-γ, corresponding to the upper proinflammatory hypercytokinemia segment. Then, immunosuppressive cytokines (IL-10, IL-1RA, TGF-β) release follows, leading the host into a condition of immune exhaustion and chronic infection, as depicted in the right immunosuppressive hypercytokinemia segment, which is also associated with impaired bacterial clearance and persistent NETs. Alterations of B, T and NK lymphocytes in terms of their numbers and quality, as well as uncontrolled activation of the PD-1 pathway, together represent patterns most likely to lead to failed antigen presentation and subsequent immune paralysis, as shown in the lower portion of the figure highlighting lymphocyte dysfunction. This depicts a time-dependent progression of systemic inflammation to immunosuppression that forms the unmodifiable pathological basis for sepsis. Created by the authors using BioRender.

immune response has been recently highlighted. lncRNA Metastasis-Associated Lung Adenocarcinoma Transcript 1 (MALAT1) modulates polymorphonuclear myeloid-derived suppressor cell (PMN-MDSC) differentiation via STAT3 phosphorylation, and its dysregulation enhances immunosuppressive functions [98]. Meanwhile, MLIP alleviates excessive inflammatory responses by regulating macrophage metabolism and oxidative stress, providing a potential target for restoring immune balance [99]. In dendritic cells, SREBF1 mediates lipid metabolism reprogramming and endoplasmic reticulum stress, thereby driving immune paralysis and further locking the body into an immunosuppressive state [100].

The diversity of immunotypes has also been confirmed by population-based studies. Transcriptomic analysis divides sepsis into two subgroups, SRS1 (immune-suppressive type) and SRS2 (predominantly proinflammatory type). SRS1 is classically defined by low HLA-II and T cell exhaustion, and is strongly associated with lower survival rates [7]. Persistent upregulation of immune checkpoint molecules, including PD-L1–related inhibitory pathways, contributes to T cell exhaustion and impaired antiviral responses, reinforcing the immunosuppressive phenotype in sepsis [81, 101, 102].

Therapies targeting immune exhaustion have shown promise. Nicotinamide riboside supplementation has been shown to partially restore T cell function via activation of NAD^+/SIRT1 signaling pathways, restore immune response, and decrease organ injury and mortality [103]. However, in elderly patients, prolonged elevation of IL-6 and increased T cell exhaustion contribute to a more sustained immunosuppressive state that is less amenable to intervention [104]. Notably, ILC2-derived IL-13 secretion in response to PD-1 deficiency partially reversed long-term muscle weakness and increased gene expression in the skeletal muscle. This indicates a strong relationship between immunosuppression and long-term functional deficits [105].

In the escalating inflammatory cascade, the immune response shifts toward a biphasic dysregulated state characterized by concurrent hyperactivation and immunosuppression. Crosstalk between pro-inflammatory and immunosuppressive signals shapes a complex immune landscape (**Figure 2**), driving sepsis progression and the transition of host defense from coordinated activation to dysregulated exhaustion. Within this network, diverse inflammatory mediators regulate intercellular communication, immune activation, and feedback control. For example, the monocyte-associated molecules CAP-1 and IL-16 participate in cell–cell communication in sepsis and septic shock and have been proposed as potential diagnostic biomarkers [106]. Elucidating the roles of inflammatory mediators in this regulatory network may therefore provide insights into sepsis pathophysiology and therapeutic targeting.

3.3 The role of inflammatory mediators in sepsis

Inflammatory mediators in sepsis function as a double-edged regulatory network that amplifies immunity while inducing negative regulation. Pyroptosis-related extracellular vesicles (pyroEVs) carrying membrane-expressed ASC proteins attach to B cells and suppress TLR4 signaling, reducing overactivated inflammation. This indicates that vesicle-mediated interventions might be of potential therapeutic value [107]. In contrast,

ferritin promotes NETosis in an MSR receptor-dependent manner via the Peptidylarginine Deiminase 4 (PAD4)/NE/ROS pathway, resulting in aggravated cytokine storm and lung injury, marking its important pro-inflammatory role in the process [108].

Among inflammatory cytokines, higher levels of MIP1α and IL-1ra are associated with lower mortality, suggesting that some mediators may counterbalance dysregulated inflammatory responses and serve as potential therapeutic targets [109]. Dexmedetomidine exerts protective effects on neuroinflammation and blood-brain barrier disruption by promoting astrocyte α2A receptor activation, implying the dynamic homeostasis of inflammatory regulation circuits between inflammation and the nervous system [110]. The enrichment of $CD14^{+}CD16^{+}$ monocytes in the blood, on the other hand, correlates tightly with increased susceptibility, severity, and mortality in sepsis, highlighting an essential role for disequilibrium among cellular populations in immunopathology [111].

Both genetic and clinical investigations support the predictive and stratification value of inflammatory variables. TNF-α -308 AA genotype or the carriers of the A allele is associated with higher serum TNF-α levels, and may serve as a genetic predictor of inflammatory responses [112]. Plasma TNF-α levels in septic patients are about 10 times higher than those in healthy individuals [113]. The TNF-α -308G/A polymorphism and IL-6 levels do not correlate with mortality in neonatal sepsis, although the prediction capacity may be heterogeneous in the population and age [114]. Of interest, TNF-α level was significantly higher in children with positive blood cultures, particularly those with Gram-negative bacterial infection and could be another alternative marker for early intervention [115]. Moreover, elevated levels of IL-6 and IL-8, as well as of KC-like and Regulated upon Activation, Normal T Cell Expressed and Secreted (RANTES), have been observed in experimental models, demonstrating the conservation of inflammatory mediators during immunopathology and their application to clinical research [116].

Inflammatory mediators in sepsis not only drive the cytokine storm and organ injury but also provide important clues for risk prediction, patient stratification, and precision therapies. The production and immunoregulatory functions of these mediators rely on the activation of multiple cellular signaling pathways, which play critical roles in inflammatory amplification, immune imbalance, and organ damage. Within this complex signaling network, N-myc and STAT interactor (NMI) has been identified as an important regulator that integrates inflammation-related signals from multiple receptors. Studies have shown that NMI participates in immune signaling through receptors such as IFNAR1, TNFR1, and TLR family members, and is closely associated with the activation of NF-κB, MAPK, and JAK pathways, thereby promoting pro-inflammatory cytokine expression and enhancing inflammatory responses [117]. Among these pathways, Toll-like receptor (TLR) signaling represents a central mechanism linking pathogen recognition to downstream inflammatory cascades in sepsis.

4 SIGNAL TRANSDUCTION PATHWAYS IN SEPSIS

4.1 TLRs and signaling cascades

As the central sensors of the innate immune system, TLRs are essential in promoting sepsis-associated inflammatory storm and immune dysregulation. Their signaling pathways are critical not only for pathogen recognition but also for inflammasome activation, metabolic imbalance, and organ failure during sepsis. There is abundant experimental and clinical data demonstrating that TLR signaling pathways play a dual role in amplifying inflammation and mediating therapeutic intervention. Fh15 protein derived from a parasite and small molecule inhibitor TAK-242 effectively inhibit LPS-induced inflammation and tissue injury, which indicates that TLR4 can be a therapeutic target [118, 119]. In parallel, both bacterial mRNA and LPS work in concert to activate the NLRP3 inflammasome, with NLRP6 also undermining host defense by sensitizing T cells through the IL-18 release. This implies a complicated interrelation and potentiation of TLR signaling by inflammasomes [120, 121].

At the systemic level, the MyD88–NF-κB–TNF-α pathway manifests a rapid phase-specific activation pattern in response to polymicrobial infection while malignant fibrous histiocytoma amplified sequence 1 presented the features of early inhibition combined with late progression along the TLR2 pathway. Such observations allow for a temporal view of the course of sepsis [122, 123]. Furthermore, serum amyloid A (SAA) acts via TLR2/TLR4 to transform HDL into pro-inflammatory particles, enhancing vascular inflammation. The elevated expression of genes like LCN2 and ELANE also support a role of TLR pathways in the immune dysregulation [124, 125]. Of note, the contribution of TLR signaling goes beyond inflammation and affects metabolism and endocrinology. TLR4/MD2-mediated LPS suppresses the growth hormone receptor, demonstrating its distinctive role in metabolic adaptation during sepsis [126]. In contrast, the transcription factor ZBTB20 enhances tissue damage through augmented activation of NF-κB. Its depletion greatly ameliorates inflammatory injury, thus emerging as a novel therapeutic target [127].

TLR-associated signaling pathways are not only central initiators of the inflammatory storm in sepsis but also important links between immunosuppression and metabolic disorders. A comprehensive exploration of their dynamic properties and their interplay mechanisms may establish the basis for the discovery of the intact landscape of disease development, as well as novel strategic avenues for stratified management and precision intervention in sepsis.

4.2 Cytokine storm and signal amplification

Excessive cytokine production represents a hallmark of immune dysregulation in sepsis, and failure to contain signal amplification contributes to systemic inflammation and multiple organ failure. Research has indicated that TNF-α and IFN-γ may cooperatively facilitate PANoptosis depending on the JAK/STAT1/IRF1 axis, which can be responsible for the deadly inflammatory cascade in COVID-19 and sepsis [94]. The systemic inflammatory response syndrome in fetal sepsis is responsible for multiple organ dysfunctions and is directly linked to severe neonatal morbidity, sepsis-related mortality and long-term sequelae risk [128]. It has also been shown that the combined action of pro-inflammatory cytokines like TNF and IL-18 can mirror many systemic effects of sepsis, leading to pervasive rewiring in both gene transcription and cellular composition that amplifies tissue destruction [84].

In the past few years, the targeting/inhibition of inflammation-induced cell death has been proposed as one of the major advances in controlling cytokine storms. Rapamycin has been reported to alleviate endothelial injury by promoting autophagy while suppressing gasdermin D (GSDMD)/caspase-1-mediated pyroptosis and decreasing the production of pro-inflammatory cytokines IL-1β, IL-18 [129]. The caspase-1 inhibitor AC-YVAD-CMK can also ameliorate acute kidney injury (AKI) by inhibiting the NLRP1/GSDMD pathway [130]. In contrast, the MSR-dependent PAD4/NE/ROS pathway in macrophages triggers NET formation in response to high levels of ferritin and promotes inflammation and lung injury, representing a mediator of poor prognostic significance [131].

The immunological features of cytokine storms vary across diseases. Sepsis and hemophagocytic lymphohistiocytosis (HLH) are diseases of overwhelming inflammation, but in contrast to sepsis patients, HLH patients show significant overactivation of CD38high/HLA-DR$^+$ effector T cells, primarily CD8$^+$ T cells. Conversely, this phenotype is not seen in early sepsis, providing a molecular rationale for the clinical distinction between the two entities [132]. Moreover, the Epidermal Growth Factor Receptor can transactivate and induce production of TNF-α in cardiomyocytes through the TACE/TGF-α pathway. Its inhibitor has been demonstrated to potently reverse heart dysfunction in animal subjects and supports the existence of druggable signaling nodes in the myocarditis inflammation network [133].

Additional layers of regulatory axes are also being uncovered. The hepatocyte Pannexin 1 (PANX1)–IL-33 axis is triggered by the ATP–P2X7 pathway, leading to ST2$^+$ Treg infiltration. This axis acts as a negative regulator for inflammation amplification, attenuating endotoxin-induced liver injury [134]. Resveratrol inhibits the microglial NLRP3/IL-1β axis via receptor-dependent mechanisms, improving cognitive function in SAE; phosphodiesterase 10A inhibitors suppress pyroptosis and organ damage by inhibiting NLRP3 inflammasome activation, providing a new idea for neuroprotection [135, 136]. Furthermore, the natural product EGCG may ameliorate inflammation and ferroptosis by dampening RELA activity and suppressing exaggerated macrophage activation, indicating that plant-derived molecules may have therapeutic potential for inflammation-related intervention [137]. IL-6 signaling, as another core pathway, regulates microtubule dynamics through the JAK2/Ninein axis, thereby promoting Extracellular Signal-Regulated Kinase (ERK) 1/2-mediated phosphorylation of p47^{phox} and LC3-associated phagocytosis. Its deficiency leads to immune paralysis and dysfunction of monocytes/macrophages [138]. This finding not only demonstrates the dynamic changes of inflammation amplification and immune hyporesponsiveness, but also offers a novel mechanistic basis for precision intervention.

Cytokine storms drive uncontrolled inflammation and multi-organ injury in sepsis, and their cross-pathway amplification reflects the dynamic imbalance between excessive immune activation and compensatory immunosuppression. Computational modeling that captures key signaling nodes and their spatiotemporal dynamics has emerged as a valuable framework for understanding these complex inflammatory networks and identifying potential therapeutic targets. In particular, agent-based modeling (ABM) has been applied to simulate systemic immune responses and to explore fundamental mechanisms of sepsis pathogenesis [139]. Persistent immune dysregulation is accompanied by profound phenotypic and functional remodeling of immune cells, including alterations in metabolic programs, differentiation trajectories, and signaling regulation, collectively shaping the systemic landscape of immune exhaustion (**Table 2**). **Table 2** summarizes the major functional switches and signaling perturbations of key immune cell populations during sepsis, providing a cellular framework for understanding immune imbalance and setting the stage for further discussion of immune cell–specific regulatory mechanisms.

4.3 Negative feedback mechanisms and immune homeostasis

In addition to the systemic uncontrolled inflammatory response, sepsis is also characterized by the activation of various negative feedback mechanisms that serve to curb tissue damage and maintain immune homeostasis. Inhibition of NLRP3 inflammasome activation and pyroptosis by certain metabolic products of myeloid cells, including itaconate, promotes inflammatory tolerance and limits tissue damage [167]. Serum exosomes have a dynamic molecular pattern in the progression of disease and their correlation with inflammatory, coagulation and vitamin metabolism pathways makes them a new approach to integrate diagnosis and therapy [168].

Table 2. Cellular characteristics of immune dysregulation in sepsis

Cell type	Phenotypic/functional alterations	Mechanistic features	Clinical consequences	Potential interventions	References
Monocytes	Enhanced anti-inflammatory response accompanied by the induction of long-lasting innate immune memory (trained immunity)	IL-4 suppresses acute inflammation and promotes trained immunity	Increased risk of opportunistic infections, morbidity, and mortality	Apolipoprotein A1–IL-4 fusion protein nanoparticle–based targeted therapy	[140]
Dendritic cells	Reduced expression of costimulatory molecules	Cytosolic mtDNA mediates immune paralysis via activation of the STING signaling pathway	Contributes to sepsis-associated immunosuppression and worsened prognosis	Hydrolysis of mtDNA or STING deficiency reverses immune paralysis and improves prognosis	[141]
Macrophages	NET formation is initially suppressed followed by delayed clearance, leading to impaired macrophage efferocytic function	Alcohol disrupts the processes of NETosis and efferocytosis	Exacerbates sepsis-associated liver injury	Neutrophil depletion alleviates LPS-induced inflammation and hepatic injury	[142]
Neutrophils	Abnormally elevated formation of NETs	Increased Padi4 expression and enhanced citrullination of histone H3	Exacerbated organ injury, intensified inflammation, and aggravated sepsis severity	Degradation of NETs using rhDNase or PAD-4 inhibitors	[143]
Microglia	Increased NET release, blood-brain barrier disruption, and neuronal apoptosis	Nuclear translocation of PD-L1 promotes p-Y705-Stat3–driven transcription of GSDMD	Leads to SAE and memory impairment	Therapeutic strategies include anti–Gr-1 antibody, DNase I, or targeting GSDMD/PD-L1	[144]
$CD4^+$ Tregs	V-domain Immunoglobulin Suppressor of T-cell Activation (VISTA) deficiency reduces Treg abundance but enhances their activity, exacerbating inflammation	The immune checkpoint VISTA exerts protective effects by modulating Tregs	Markedly reduced survival, accompanied by aggravated organ injury and inflammation	Strategies include enhancing VISTA expression or adoptive transfer of $VISTA^+$ Tregs	[145]
Macrophages	Enhanced aerobic glycolysis and overactivation of the inflammasome	PKM2 promotes inflammasome activation via modulation of Eukaryotic translation initiation factor 2α kinase 2 (EIF2AK2) phosphorylation	Leads to lethal endotoxemia and sepsis	Pharmacological or genetic inhibition of PKM2 or EIF2AK2	[146]
T lymphocytes	Reduced lymphocyte apoptosis and suppressed cytokine production by macrophages	The VISTA immune checkpoint transmits inhibitory signals	Enhanced bacterial clearance, mitigated organ injury, and improved survival	Treatment with high-affinity anti–VISTA antibody (MH5A)	[147]
Cardiomyocytes	Significantly increased succinylation of Voltage-Dependent Anion Channel 2 (VDAC2) protein	Succinyl-CoA induces succinylation at VDAC2 K46, triggering ferroptosis	Leads to SIMD	Therapeutic interventions include ND-630 or mitochondria-targeted nanoparticle TPP-AAV	[148]
Renal tubular epithelial cells (HK-2 cells)	Enhanced aerobic glycolysis, increased lactate production, and suppressed autophagy	Lactate inhibits autophagy by downregulating the SIRT3/p-AMPK pathway	Leads to SI-AKI	Treatment with the aerobic glycolysis inhibitor 2-DG	[149]
Pulmonary epithelial cells	Reduced Skp2 expression and impaired membrane localization of Solute Carrier Family 3 Member 2 (SLC3A2)	MEK/ERK pathway suppresses Skp2, decreases SLC3A2 ubiquitination, and triggers ferroptosis	Leads to sepsis-induced ALI	Intravenous administration of lipid nanoparticles (LNP) encapsulating Skp2 mRNA	[89]
BM cells	Persistent production of proinflammatory cytokines and hyperresponsive phenotype	METTL14 deficiency reduces SOCS1 m^6A methylation, leading to decreased SOCS1 levels	Bacterial infection results in high mortality	Forced expression of SOCS1 rescues the hyperresponsive phenotype	[150]

Hepatocytes	Exacerbated cytokine storm and dysregulated immune response	PANX1 positively regulates IL-33 synthesis via the ATP-P2X7 pathway	Worsens endotoxemia and post–liver transplant hepatic injury	Therapeutic approach involves recombinant IL-33 targeting ST2$^+$ Tregs	[134]
Pulmonary endothelial cells	Elevated Histone H3 lysine 14 lactylation (H3K14la), leading to endothelial activation and ferroptosis	Glycolysis-derived lactate induces H3K14la, promoting expression of ferroptosis-related genes	Leads to sepsis-induced ARDS	Inhibition of glycolysis or H3K14la to block ferroptosis	[151]
Pulmonary epithelial cells	Upregulated Olfactomedin 4 (OLFM4) expression suppresses LPS-induced proinflammatory responses	OLFM4 inhibits the LDHA/NF-κB pathway by reducing ROS and HIF1α levels	Modulates sepsis-associated ARDS	Therapeutic approaches include recombinant OLFM4 or OLFM4 overexpression	[152]
T cells	Increased T cell infiltration and decreased dendritic cell infiltration	Lysosomal metabolic dysregulation is associated with an imbalanced immune microenvironment	Contributes to sepsis and ARDS	CTSO and HLA-DQA1 may serve as novel diagnostic and therapeutic targets	[153]
Pulmonary fibroblasts	Suppressed Peroxisome Proliferator-Activated Receptor Gamma Coactivator-1 Alpha (PGC-1α) expression and increased release of mitochondrial DNA-containing microparticles	Mitochondrial dysfunction drives mitochondrial DNA-containing microparticle–induced MMT	Facilitates the progression of SAPF	Activation of PGC-1α alleviates fibrosis, representing a potential therapeutic strategy	[154]
Renal tubular epithelial cells	Attenuated ferroptosis with increased GPX4 expression	NRF2 nuclear translocation upregulates GPX4 expression, inhibiting ferroptosis	Alleviates SI-AKI	Treatment with melittin, the main component of bee venom	[155]
CD64$^+$ immature neutrophils (CD123$^+$ and PD-L1$^+$ subsets)	Two novel subsets emerge with impaired activation and phagocytic functions	The proportion of CD123$^+$ neutrophils correlates with clinical severity	Useful for specific diagnosis of sepsis and differentiation from other inflammatory conditions	Rapid diagnosis and therapeutic guidance via blood testing using seven markers	[156]
Neutrophils (CXCR2$^+$ and CD274$^+$IL1RN$^+$ subsets)	Markedly increased neutrophils exhibiting an immunosuppressive phenotype	Mechanistic feature: developmental trajectory from CXCR2$^+$ to CD274$^+$IL1RN$^+$ subsets	Leads to pulmonary immunosuppression, increasing the risk of secondary infections and mortality	CXCR2 blockade reduces PD-L1$^+$ neutrophils and improves survival	[157]
CD8$^+$ T cells	Increased platelet MHC-I expression with enhanced antigen cross-presentation capacity	Platelets modulate CD8$^+$ T cells via MHC-I–mediated antigen cross-presentation	Impairs CD8$^+$ T cell function and correlates with sepsis mortality	Targeting platelet MHC-I improves CD8$^+$ T cell responses and survival	[158]
T lymphocytes	Decreased neutrophil apoptosis and increased T lymphocyte apoptosis	HMGB1 upregulates neutrophil PD-L1 via TLR2, engaging T cell PD-1	Leads to immunosuppression and sepsis-induced lung injury	Therapeutic interventions include GA or the TLR2 inhibitor C29	[102]
ILC2s	ILC2 activation with increased secretion of IL-5 and IL-13	IL-33 induces IL-5 secretion by ILC2s, protecting cardiomyocytes	Alleviates sepsis-induced cardiac dysfunction and myocardial injury	Therapeutic strategies include IL-33 supplementation or harnessing ILC2 function	[159]
Neutrophils	Elevated HMGB1 lactylation with increased formation of NETs	Lactate promotes HMGB1 lactylation via macrophage-derived exosomes, activating the cGAS/STING pathway	Exacerbates SA-AKI	HMGB1 may serve as a potential therapeutic target for SAKI intervention	[160]
NK cells	Increased monocytes and decreased inactive NK cells	Coagulation-related genes are involved in immune activation, antigen processing, and other pathways	Contributes to sepsis-induced organ dysfunction	FCER1G and FYN may serve as diagnostic biomarkers and therapeutic targets	[161]

Macrophages	Low expression of ferroptosis-related gene Lipin 1 (LPIN1) with increased macrophage infiltration	LPIN1 downregulation is associated with inflammation and immune response pathways	Leads to poor prognosis and reduced survival in sepsis patients	LPIN1 may serve as a prognostic biomarker and potential therapeutic target	[162]
T cells	Elevated soluble urokinase-type plasminogen activator receptor (suPAR) levels with increased renal T cell infiltration, exacerbating inflammation	suPAR promotes T cell–mediated renal inflammation by upregulating chemokines	Leads to more severe SI-AKI, increasing the need for RRT and mortality risk	suPAR deficiency ameliorates SI-AKI, indicating it as a potential therapeutic target	[163]
Hippocampal neurons	Upregulated Fgr expression associated with neuroinflammation, oxidative stress, and mitochondrial dysfunction	Fgr inhibits SIRT1 activity, subsequently affecting the PGC-1α pathway	Leads to SAE and cognitive-emotional dysfunction	Fgr kinase inhibitors confer protection by activating the SIRT1/PGC-1α pathway	[164]
Hepatocytes	Marked upregulation of S100A8/A9 with hepatic dysfunction and mitochondrial impairment	S100A9 inhibits the AMPK pathway, affecting fatty acid and glucose metabolism	Leads to sepsis-induced ALI	S100A9 inhibitor Paquinimod (Paq) can prevent liver injury and mortality	[165]
Liver macrophages	Hepatocyte mitochondrial structure and energy supply are impaired, accompanied by altered TREM2 expression	TREM2-deficient macrophages release exosomes containing miR-106b-5p, inhibiting Mfn2	Nonalcoholic fatty liver disease (NAFLD) increases susceptibility to sepsis and mortality	Overexpression of TREM2 in liver macrophages improves hepatic energy supply and sepsis outcomes	[166]

Perturbations in immune homeostasis are associated with disease progression. Silencing Cystatin C accelerates LPS-induced inflammation by inhibiting autophagy and increasing caspase-11 expression, which enhances NLRP3 inflammasome activation [169]. T-cell lymphoma breakpoint-associated target genes and Sestrin 2 boost host resistance and disease tolerance by inducing autophagy and mitochondrial quality control, which increases clearance of bacteria but attenuates organ damage [136, 170]. These data demonstrate that autophagy-associated pathways are central hubs for fine-tuning inflammation versus tissue protection.

In terms of signaling regulation, S100A10 inhibits the TLR pathway to downregulate excessive inflammation, and lack of it increases immune damage [171]. Membrane-bound MHC I molecules feed back to suppress TLR signaling through the Fps–SHP-2 pathway to provide self-restricted protection of innate immunity [172]. Meanwhile, immunosuppression may stem from imbalanced endogenous negative feedback, whereby SAMSN1 promotes an immune-suppressive state that can be reversed upon its depletion [173]. APE1 deficiency impairs macrophage phagocytic ability via the GSK3β/Nrf2 axis, contributing to bacterial infection and immune defects [174]. Notably, TSLP signaling serves as a central protective factor for myeloid cells. Its absence does not impair bacterial eradication, but exacerbates systemic inflammation and organ injury. By contrast, the survival of mice is significantly enhanced by TSLP-mediated suppression of hyperactivation of myeloid cells [8].

While dysregulated signaling cascades orchestrate the acute immune and metabolic responses in sepsis, inter-individual variability in these pathways is profoundly influenced by inherited genetic variations. Genome-wide association studies have identified several low-frequency variants associated with reduced 28-day survival in sepsis, including a missense variant in SAMD9 (hazard ratio [95% CI]=1.64 [1.37–6.78], $P=4.92\times10^{-10}$), a gene implicated in inflammatory responses that may mediate tissue injury [175]. In parallel, integrative genetic analyses incorporating Mendelian randomization have identified multiple candidate genes significantly associated with sepsis susceptibility, several of which remained robust after sensitivity analyses. These findings collectively highlight the contribution of host genetic variation to disease susceptibility and prognosis [176]. Thus, integrating host genetic architecture into the framework of signaling networks provides an essential dimension for understanding disease heterogeneity and differential clinical outcomes. Importantly, many of these genetically influenced pathways also generate measurable molecular signatures that are increasingly investigated as emerging biomarkers for diagnosis, risk stratification, and prognosis in sepsis.

5 HOST GENETIC FACTORS AND SUSCEPTIBILITY TO SEPSIS

5.1 Genetic polymorphisms and individual differences

Host genetic variations play a critical role in sepsis susceptibility and outcomes, affecting various aspects ranging from pathogen recognition and immune responses to metabolic homeostasis and organ injury. Matrix metalloproteinase (MMP) 8 rs11225395 G/G genotype is associated with sepsis susceptibility, and MMP1 and MMP3 are not only linked to

bacterial and viral infection susceptibility but also to agent-specific levels of severity. This indicates that the genetic variation of the host plays a role in pathogen-specific patterns of immune defense [177]. Gene-based analyses have also demonstrated that sepsis-2 and the Sepsis-3 criteria not only have a polygenic overlap but also possess unique molecular attributes. Their multigene risk scores also have a high predictive value, suggesting that sepsis susceptibility usually results from the combined effect of multiple small-effect polymorphisms [178].

The interplay of genetic susceptibility and environmental factors has been given more attention in research. For instance, genetic insomnia is positively associated with sepsis risk, and this link is in part explained by cardiac metabolism pathways involving heart function. This indicates that sleep disturbances may be a targetable genetic risk factor [179]. Alterations of the coagulation and complement cascades contribute significantly to the disease course. Polymorphisms in the THBD gene are strongly associated with AKI risk and death [180]. A gain-of-function variant in cholesteryl ester transfer protein (CETP) is associated with increased acute sepsis mortality, whereas genetic evidence for decreased CETP function, together with pharmacological CETP inhibition in humanized mouse models, suggests potential survival benefits through the preservation of HDL-C levels [181]. Different variants of complement C5 can either reduce susceptibility or, conversely, accelerate disease progression by enhancing C5a generation and the release of pro-inflammatory mediators [182].

Immunity- and metabolism-related pathways also serve as hub nodes that regulate the disease. The VDR Fok I polymorphism is a strong risk factor for sepsis, and it correlates positively with lower vitamin D status, thus indicating that the vitamin D metabolic axis may represent an important host regulatory pathway [183]. In patients with diabetes, an association was found between Monocyte Chemoattractant Protein-1 (MCP-1) rs1024611 G allele and susceptibility to sepsis, which may be attributed to upregulated expression of MCP-1 and TNF-α [45]. High-risk allele combinations of TNFA rs1800629 and TLR4 rs4986791 in trauma patients are associated with significantly increased risk of sepsis, considering that TLR4 is a prognostic variant [184]. Moreover, although the reported associations of certain polymorphisms in genes including CHRNA7, NR5A2 and SUFU with sepsis-associated acute kidney injury (SA-AKI) have not shown genome-wide significance, these findings underscore the influence of host genetic differences on disease phenotype [185]. Polymorphisms in inflammation-related genes provide broader mechanistic insights. The elevated serum and urinary IL-10 levels in carriers of the IL-10 rs1518111 T allele are strongly associated with the development of SA-AKI, demonstrating that both inflammatory factor levels as well as genetic susceptibility play critical roles in the risk of organ damage [186]. Furthermore, PCSK9 loss-of-function not only increases organ injury by altering lipoprotein metabolism and removal of endotoxins but also impairs endothelial function by regulating Angpt-1, thereby aggravating septic pathology [187].

Susceptibility to sepsis is not monogenic but due to the combined action of multiple pathway variants. Such effects include inflammation control, immunological response, coagulation deregulation and metabolic perturbation. A comprehensive investigation of the dynamic properties and interaction mechanisms in these genetic networks can not only help uncover the molecular pathogenic basis for sepsis susceptibility, but also serves as an essential framework for developing risk prediction models and precision intervention strategies.

5.2 Gene expression regulation and inflammatory response

Inflammatory dysregulation in sepsis does not ensue from a single signal but rather represents a systemic disorder arising as a consequence of the perturbation of transcriptional homeostasis, abnormal epigenetic changes and malfunctioning noncoding RNA networks. It has been reported that the disproportion of expression of TLR4 and GRP78 is closely related to patient prognosis, indicating their dynamic properties during abnormal inflammatory responses and their potential to serve as monitoring parameters [188]. At a much deeper level, however, the transcription factor PU.1 induces MDSC differentiation and aggravates immune suppression via lncRNA Hotairm1 and its related epigenetic modification network. This connects transcription factors, epigenetic marks and immune dysregulation in a coordinated manner [189]. Additionally, the function of m^6A methylation in immune cell infiltration and Th17 differentiation is receiving more attention. Master factors, such as METTL16 and IGF2BP1, were demonstrated to be involved in the progression of the late inflammatory phase, strongly suggesting the utility of epigenetic manipulation [190].

Multivariate complexity of gene regulatory networks in organ injury varies across different organs. Adenosine-uridine RNA editing of neuronal gene Grik2 and Flna in SAE are involved in the development of cognitive deficits. This phenomenon exhibits age- and sex-specific traits [191]. Research on natural compounds has discovered new ways of modulating inflammation and oxidative stress.Vitexin relieves lung injury in part through the SNHG1/DNA methyltransferase (DNMT) 1/miR-495 axis, while Gossypol alleviates myocardial injury via HDAC inhibition and histone acetylation remodeling [192, 193]. Furthermore, exosomes and non-coding RNAs were found to be implicated in immune reprogramming. Microvesicles derived from patients can deliver DNMT1/3A, which results in hypermethylation of the TNF-α promoter and immunosuppression [194]. circMAPK1 promotes NLRP3 inflammasome activation through modulating demethylation, which worsens lung injury [195]. On the other hand, miR-221-5p enhances inflammatory responses by inhibiting mitophagy and promoting organ damage as well [196].

The widespread involvement of m^6A modification in sepsis-induced multiple-organ injury is noteworthy. METTL3-triggered methylation has been reported to promote liver injury progression, but in the heart, lncRNA and mRNA expression differences lead to functional decline through the TNF and PI3K-Akt signaling pathways [197, 198]. In AKI, the NF-κB/miR-376b/NFKBIZ negative feedback system not only controls inflammation and cell death but also shows the diagnostic significance of urinary miR-376b [199].

Gene regulatory networks of sepsis have global characteristics with complex coupling between multiple hierarchical levels, pathways, and organs. In this dynamic, feedback-loop-driven environment, inflammation potentiation and immune dysfunction maintain a parallel course, leading to multi-organ failure. More detailed exploration of these mechanisms not only uncovers the molecular basis of sepsis but also provides a basis for precision therapy and personalized medicine.

5.3 Clinical significance of emerging biomarkers

Advances in novel biomarkers are reshaping the diagnosis and treatment of sepsis. Evidence from the endocrine and metabolic aspects suggests that using T3 within the 60–80 ng/dl range is related to lower incidence rates of Persistent Inflammation, Immunosuppression, and Catabolism Syndrome (PICS), whereas profound T3 deficiency in combination with high Sequential Organ Failure Assessment (SOFA) scores predicts poor outcomes [200]. Metabolomic studies have indicated the potential causal role of dysregulation of the glycolysis/pyruvate pathway in the risk and prognosis of sepsis, which can lay a strong foundation for developing metabolic biomarkers for early diagnosis [201].

Genetic and immune biomarkers also show significant clinical utility. In patients, individuals with the LAG-3 rs951818 AA genotype were found to have a reduced risk of death, suggesting their potential value as a prognostic genetic marker [202]. Furthermore, molecular fingerprints of IRRGs perform well in identifying high-risk individuals and monitoring immune status, paving the way for precise intervention [203]. Synergistic detection of inflammatory mediators has also improved clinical prediction models. IL-10 levels integrated with the National Early Warning Score (NEWS) score further enhances sensitivity in early detection of sepsis risk, and the combined measurement of urinary CRP, α1-acid glycoprotein and SAA dramatically improves discrimination between sepsis and SIRS [204, 205]. In addition, easy-to-obtain markers such as NLR, PLR and LMR have been shown to be clinically useful in the early postoperative period [206]. Furthermore, the T-cell CD69 ratio combined with PCT can accurately differentiate between gram-negative and gram-positive sepsis and also has predictive value [207].

The range of biomarkers is constantly increasing based also on multi-omics and non-coding RNA research. Excessive expression of CEACAM8, MPO and RETN has been demonstrated to be closely correlated with disease progression, hence offering potential as diagnostic urinary biomarkers [208]. In addition, the correlation between PGK1 and immune cell infiltration and inflammation regulation also demonstrates its potential as a new diagnostic index [209]. Furthermore, the roles of ncRNAs have become a subject of much interest. LINC01278 serves as a sponge of miR-451a to weaken inflammation and organ injury, and MEG3 rs7158663 GG genotype dramatically elevates genetic susceptibility and adverse outcome risk through promoting the overflow of inflammatory factors [210, 211]. Together, these findings depict a multidimensional biomarker network encompassing metabolic, genetic, immune, and non-coding RNA pathways. This network not only deepens our understanding of the complex molecular landscape of sepsis but also facilitates improved patient stratification and immune status monitoring. Importantly, these advances provide a critical basis for the development of precision medicine approaches and targeted therapeutic strategies, paving the way for the emerging immunomodulatory and adjunctive therapies discussed in the following section.

6 EMERGING THERAPEUTIC STRATEGIES AND CLINICAL CHALLENGES

6.1 Targeted immunomodulatory therapies

Recently, the investigation of therapeutic immune modulation has become a major trend in sepsis research. Promising approaches such as small molecules and targeted agents were discussed. For example, PLX5622 prevents neuronal injury and synapse loss by suppressing microglial activation, attenuating SAE [212]. However, the PSMA4-associated protein degrader has been identified as a new target for therapy and may reduce the risk of sepsis development [213]. Among traditional pharmacological strategies, glucocorticoids in combination with fludrocortisone have been evaluated in a component network meta-analysis of 33 randomized controlled trials including 9,898 patients, in which this combination was associated with reduced short-term mortality (RR 0.89) and improved long-term mortality in adults with sepsis and septic shock, highlighting their potential as an adjunctive therapy [214]. In addition, a prospective multicenter study of granulocyte–monocyte adsorption apheresis in 82 patients with sepsis demonstrated a significant reduction in SOFA score at 7 days and a 28-day mortality rate of 7.8%, supporting its safety and potential adjunctive benefit [215].

Moreover, the profiling of immune-cell functions has also identified additional possibilities for therapeutic intervention. Single-cell immune profiling further demonstrates the heterogeneity of immune cells in PICS post-sepsis, which offers

potential individual targets for risk evaluation and precision treatment [216]. At the molecular pathway level, caspase-11/GSDMD was found to regulate NET generation via neutrophil activation, which indicates GSDMD may be a therapeutic target for sepsis treatment [217]. By contrast, HMGB1 can act as a ligand for Tim-3 to elicit immunosuppression via inhibition of NF-κB signaling in $CD4^+$ T cells, thus representing a potential translational impact [218]. Metabolism-regulating molecules like NLRC3 mediate immunosuppression by shaping macrophage metabolism, and the targeted intervention can possibly restore the immune derangement during sepsis [219]. A $CXCR2^+$ neutrophil subset has been demonstrated to promote pulmonary immune dysregulation, and its blockade could help in mitigating lung injury and improving outcomes [157]. Furthermore, ulinastatin also exerts unique immunomodulatory effects through dual regulation of neutrophils and MDSCs [220].

Platelets, T cells and metabolic pathways have also been identified as potential therapeutic targets. Platelets modulate the function of $CD8^+$ T cells through MHC-I-mediated cross-presentation of antigens, and this newly described mechanism offers new opportunities in immunotherapeutic approaches [158]. IFN-γ can also restore immunosuppression by promoting a switch to the Warburg effect through the PI3K/Akt/mTOR pathway, and Inducible T-cell Co-Stimulator (ICOS)-Fc bimodulates the ICOS–ICOSL signaling axis, significantly ameliorating systemic inflammatory response syndrome and multiple organ injuries [221, 222]. Interventions on cellular and molecular levels still increase. Hematopoietic stem cell infusion, for instance, can increase MDSCs and suppress inflammation to improve survival; low-dose esmolol attenuates T cell apoptosis and normalizes Th1/Th2 imbalance by acting on the Akt/Bcl-2/Caspase-3 and ERK1/2 pathways [223, 224].

Promising microorganism and non-coding RNA-mediated therapies are also emerging. Tripeptide Arginine-Lysine-Histidine (RKH), derived from Akkermansia muciniphila (AKK), reduces inflammation and organ damage by inhibiting TLR4 signaling [66]. Nicorandil alleviates myocardial injury through mediating ferroptosis of the TLR4/SLC7A11 pathway; low-dose mycophenolate mofetil reinforces anti-bacterial activity of macrophage, hence promoting bacterial clearance for survivors [225, 226]. Molecularly, miR-150 supplementation can reverse the immunosuppressive role of MDSCs as an innovative intervention strategy for late-stage sepsis; additionally, in preclinical caecal ligation and puncture (CLP) models, umbilical cord–derived MSCs and their conditioned media were shown to mitigate organ injury, enhance bacterial clearance, reduce serum lactate and pro-inflammatory cytokine levels, and improve survival when administered within a defined therapeutic window [227, 228].

The paradigm of sepsis immunoregulation is shifting from single-target interventions toward systemic reprogramming strategies. Emerging evidence indicates that sepsis induces metabolic reprogramming and dysregulated IL-17 production in $CD4^+$ T cells, leading to persistent immune dysfunction and impaired host defense after recovery [9]. These findings highlight the critical role of immunometabolic imbalance in sepsis pathophysiology and have stimulated increasing interest in therapeutic strategies targeting immune and metabolic dysregulation.

Within this evolving framework, diverse therapeutic approaches converge on a common objective: restoring immune homeostasis and cellular energy balance. Current strategies include cell-based therapies, nanodelivery systems, metabolic reprogramming, and targeted immunomodulation (see **Table 3**), reflecting a growing trend toward multi-target and system-level interventions. For example, mitochondrial transplantation has been shown to reverse sepsis-induced metabolic suppression and restore immune cell bioenergetics, promoting recovery of oxidative metabolism and biosynthetic capacity [253]. Beyond metabolic modulation, therapeutic strategies targeting inflammatory responses and oxidative stress are also emerging as promising approaches in sepsis management.

6.2 Novel approaches in antioxidant and anti-inflammatory therapy

In recent years, different therapeutic options for sepsis have emerged, and several promising new strategies have appeared with great clinical potential. Plasma exchange has been evaluated in a meta-analysis of five controlled trials, in which it was associated with reduced all-cause mortality in adults with severe sepsis (OR 0.54), although heterogeneity among studies was substantial and larger randomized trials are still required to confirm these findings [254]. Early enteral nutrition has emerged as a promising and generally safe therapeutic strategy in critically ill patients with sepsis and septic shock. A systematic review including five randomized controlled trials (n=442) and ten non-randomized studies (n=3,724) suggested that early enteral nutrition was associated with fewer days of mechanical ventilation and lower SOFA scores during follow-up, although no statistically significant reduction in in-hospital mortality was observed [255]. The immunomodulator glatiramer acetate (GA) has demonstrated anti-inflammatory and immunomodulatory effects in a CLP murine model of sepsis. Acute administration (1–2 mg/kg) significantly improved 72-hour survival and reduced pro-inflammatory cytokine levels, suggesting potential therapeutic relevance that warrants further clinical investigation [256].

In anti-infective therapy, the combination of azithromycin with exosomes provides a new therapeutic agent for sepsis by boosting infection clearing and regulating inflammation [257]. Nutmeg extract attenuates platelet desialylation and clearance by suppressing sepsis-elicited sialidase activity, indicating its prospect for use in the treatment of sepsis and sepsis-associated

Table 3. Innovative intervention strategies and their research progress

Intervention type	Representative drug/method	Mechanistic target	Clinical progress/trial stage	Limitations	References
Cell therapy	Autologous neutrophils subjected to cryo-shock	Broad-spectrum neutralization of inflammatory cytokines and endotoxins	Preclinical study	Further clinical validation required for efficacy and safety	[229]
Nanomedicine	NAD(H)-loaded nanoparticles	Supplementation of NAD(H) to enhance energy supply and suppress inflammation	Preclinical study	Biosafety of nanomaterials and their clinical translational potential require validation	[230]
Immunometabolic modulator	MSM	Promotes macrophage M2 polarization via the lactate-H3K18la pathway	Preclinical study	Mechanistic insights require further elucidation, and clinical efficacy remains to be validated	[231]
Metabolites	FMT and SCFAs	Modulates gut microbiota, inhibits pyroptosis, and protects intestinal barrier	Preclinical study	Safety and efficacy in humans require clinical validation	[232]
Immune checkpoint inhibitor	Nivolumab combined with meropenem	Inhibits the PD-1 pathway and reverses T cell exhaustion	Mathematical modeling simulations; preclinical stage	Efficacy depends on precision medicine; ineffective under high pathogen load	[233]
Blood purification	CVVH	Removes inflammatory mediators and improves immune and endothelial function	Small randomized controlled trial	Did not reduce 28-day mortality or shorten hospital stay	[234]
Stem cell therapy	MSCs and their exosomes	Targets the MALAT1 pathway via delivery of miR-26a-5p	Preclinical study	Mechanism is complex; clinical safety and efficacy require validation	[235]
Immunomodulatory therapy	C1q-blocking antibody and PLX5622	Inhibits C1q-mediated synaptic pruning and microglial activation	Preclinical study	Clinical validation has not been conducted; efficacy remains to be evaluated	[212]
Complement system targeting	C5a receptor 1 (C5ar1) gene deficiency	Blocks C5ar1 to reverse immunosuppression	Preclinical study	Based on gene deficiency models; drug development and efficacy require validation	[236]
Novel small-molecule drug	Songorine	Activates the Nrf2/ARE pathway to promote mitochondrial biogenesis	Preclinical study	Plant-derived compound; clinical translational potential remains to be validated	[237]
Herbal medicine	Rhodiola rosea extract	Inhibits the PI3K-AKT pathway to alleviate inflammation and oxidative stress	Preclinical study	Complex composition; mechanism requires further elucidation, and clinical efficacy remains to be validated	[238]
Drug repurposing	Montelukast	Inhibits downstream inflammatory and oxidative stress pathways such as LTB-4	Preclinical study	Currently at the animal experiment stage; clinical efficacy is unknown	[239]
Natural product	Isoflavone osajin	Inhibits the IL-33/LPO/8-OHdG/caspase-3 pathway	Preclinical study	At the animal experiment stage; clinical efficacy remains to be validated	[240]
Protein-delivery nanomedicine	MF-LNP encapsulating SOD/CAT	Efficiently delivers antioxidant enzymes to scavenge ROS	Preclinical study	Safety and clinical translational potential of the novel delivery system require validation	[241]
Therapeutic strategies targeting METTL3	METTL3 inhibitors or knockdown approaches	Inhibits METTL3 to block m^6A-dependent ferroptosis	Preclinical study	Clinical validation has not been conducted; safety and efficacy remain to be evaluated	[242]
Drug repurposing	Dexmedetomidine	Activates astrocytic α2A adrenergic receptors	Preclinical study	Mechanism requires further elucidation; clinical efficacy remains to be validated	[110]
Probiotic-derived peptides	AKK or its derived tripeptide RKH	Acts as an endogenous TLR4 antagonist to block TLR4 Signaling	Preclinical study	Human trials have not been conducted; clinical translational efficacy remains to be validated	[66]

Microbial therapy	Lactobacillus rhamnosus GG	Modulates gut microbiota and reduces systemic inflammation via macrophages	Preclinical study	Mechanism is indirect, no direct effect on the brain; clinical efficacy remains to be validated	[243]
Herbal medicine	Gedunin	Inhibits the HMGβ1/NLRP3/NF-κB signaling pathway	Preclinical study	At the animal experiment stage; clinical translational potential remains to be validated	[244]
Monoclonal antibody	Humanized anti-citrullinated histone H3 monoclonal antibody	Blocks the CitH3-TLR2 signaling axis to inhibit NETosis and pyroptosis	Preclinical study	Has not entered clinical trials; safety and efficacy remain to be validated	[245]
Uridine supplementation therapy	Uridine as a metabolic supplement	Activates the Nrf2 signaling pathway to inhibit macrophage ferroptosis	Preclinical study	At the animal experiment stage; clinical translational potential remains to be validated	[246]
Targeted immunomodulation	Disulfiram or GSDMD gene knockout	GSDMD/caspase-11 pathway	Preclinical study	At the animal experiment stage; clinical efficacy remains to be validated	[217]
Neuroimmune modulation	Electroacupuncture	Promotes M2 macrophage polarization by upregulating spermidine and ODC expression	Preclinical study	At the animal experiment stage; clinical translational potential remains to be validated	[247]
Physical therapy	Nurse-led, goal-oriented pulmonary physical therapy	Improves pulmonary ventilation and drainage via physical means; no clear molecular target	Completed clinical controlled studies showing improvement in clinical outcomes	Implementation depends on a specialized nursing team	[248]
Photodynamic therapy	Aloe-emodin-mediated photodynamic therapy	Inhibits the expression and activity of gram-positive bacterial toxins such as cytolysins, hemolysins, and pneumolysins	Preclinical study	Clinical efficacy remains to be validated	[249]
Gut microbiota modulation	Metformin	Modulates gut microbiota and alleviates colonic barrier dysfunction	Preclinical study	At the animal experiment stage; clinical translational potential remains to be validated	[250]
Protein supplementation	Vitamin D binding protein	Alleviates oxidative stress and inhibits the JNK signaling pathway	Preclinical study	Mechanism requires further elucidation; clinical efficacy remains to be validated	[83]
High-dose antioxidant therapy	High-dose intravenous vitamin C	Antioxidant effect (specific molecular target not clearly defined)	Ongoing multicenter randomized phase III clinical trial	Trial is ongoing; efficacy and safety outcomes are unknown	[93]
Natural product	Curcumin	Reduces free radicals, enhances antioxidant enzymes, and inhibits iNOS	Preclinical study	At the animal experiment stage; clinical efficacy remains to be validated	[251]
Kinase inhibition	Fgr kinase inhibitor	Fgr kinase → SIRT1/PGC-1α signaling pathway	Preclinical study	At the animal experiment stage; clinical efficacy remains to be validated	[252]

thrombocytopenia [258]. In addition, oXiris continuous hemofiltration adsorption was evaluated in a retrospective cohort of 90 patients with sepsis or septic shock. Significant improvements in hemodynamic parameters, lactate levels, inflammatory biomarkers (procalcitonin and IL-6), and SOFA scores were observed at 12 and 24 hours post-treatment. Pre-treatment SOFA score, percentage SOFA reduction, and age were identified as independent predictors of ICU mortality. Although these findings suggest potential benefits in organ function stabilization, prospective randomized trials are needed to confirm survival impact [259].

Recently, immunomodulatory therapy has become an innovative approach to the treatment of sepsis. For instance, hPF4 complexes with cfDNA and NETs to suppress thrombosis and endothelial injury, which could be potential targets for immune therapy in sepsis [260]. Codonopsis Pilosula Extract reduces immune organ damage in septic rats, and keeps the immune homeostasis by regulating BCR signaling pathway and glycerophospholipid metabolism, highlighting its future potential for sepsis immunomodulation [261]. For sepsis-related lung injury, montelukast mitigates lung tissue damage, and its administration effectively ameliorates the polymicrobial sep-

sis-induced increase in inflammatory mediators and oxidative stress, thereby providing protection [239]. Gedunin exhibits a marked protective effect against oxidative stress and inflammation by suppression of HMGB1/NLRP3/NF-κB signaling pathway in septic mouse models, which provides valuable implications for its promising therapeutic applications [244]. Songorine can act through Nrf2/ARE and Nrf1 signaling pathways to enhance mitochondrial biogenesis, ameliorate cardiac function, and provide protective effects against sepsis-induced myocardial injury [237].

In the treatment of kidney injury, Osajin mitigates sepsis-induced acute kidney injury (SI-AKI) by suppressing the IL-33/Lipid Peroxidation (LPO)/8-hydroxy-2'deoxyguanosine (8-OHdG)/caspase-3 pathway and promoting antioxidant defense, indicating its possible use in sepsis therapy [240]. The deletion of MALAT1 controls epigenetic regulation, activates the methionine cycle, and improves antioxidative activity, which may provide a new direction for the treatment of sepsis [262]. Moreover, betaine remarkably decreases the levels of inflammatory and oxidative stress markers, improves gas exchange and imaging findings as well as protective effects against sepsis-induced ARDS [263].

Regarding cardiac preservation, the Mitochondria-targeted 2,2,6,6-tetramethylpiperidine-1-oxyl (MitoTEMPO) enhances mitochondrial biogenesis and protects against sepsis-induced renal injury by decreasing mROS and IL-1β [264]. MSCs and their exosomal miR-26a-5p delivery target and suppress MALAT1 to alleviate hepatocyte death and oxidative stress, and are protective against sepsis-induced ALI [235]. Furthermore, matairesinol is a potent neuroprotectant against sepsis-induced brain damage by activating the AMPK/Nrf2 signaling pathway and blocking MAPK/NF-κB pathways to reduce both neuroinflammation and oxidative stress [265].

In terms of neuroprotection, quercetin also markedly mitigates SAE through the suppression of the CXCL2/CXCR2 axis, associated microglial activation, and neuronal ferroptosis [266]. Eriocitrin protects against SA-AKI through the activation of Nrf2 pathway, mitochondrial dynamics, and decrease in inflammation and oxidative stress [267]. Taohe Chengqi Decoction can effectively suppress the inflammatory response and correct immune imbalance in sepsis via mediation of several signaling pathways, such as MAPK and STAT3, thereby representing a potential therapeutic strategy for sepsis [268].

Emerging immunomodulatory and antioxidant therapeutic strategies have shown great potential in the clinical management of sepsis, particularly in improving patient outcomes. Further elucidating these therapeutic mechanisms and advancing their clinical translation will pave the way for personalized treatment and precise interventions in sepsis.

6.3 Application of personalized medicine in sepsis

Innovative sepsis management approaches have increasingly demonstrated the intimate interplay of patients' immune responses, metabolic alterations and clinical outcome. Dynamic fluid monitoring is one of the most efficient methods for improving the prognosis of patients with septic shock [269]. In addition, enhanced external inspection and monitoring could shorten antibiotic therapy and fluid resuscitation durations effectively to optimize the clinical treatment program significantly [270]. Furthermore, the low expression of miR-146b-5p in patients with sepsis is closely related to a poor prognosis. When the APACHE II score is used in conjunction, it greatly increases the accuracy of the diagnosis. Similarly, tracking the dynamics of lymphocyte subsets strongly advocates for individualized treatment [271]. Immune heterogeneity of sepsis has been demonstrated by SRS molecular typing. This result offers an important theoretical basis for precision medicine, and indicates that personalized treatment guided by the host immune response status may be the direction for improving sepsis prognosis [7].

To better stratify sepsis, studies using coagulation biomarkers have identified four distinct sepsis phenotypes. Among these phenotypes, patients with high fibrin degradation products/D-dimer levels may be more responsive to thrombomodulin therapy, supporting the development of personalized medicine approaches for sepsis [272]. The underlying mechanisms of immune diversity have also been further revealed by studies highlighting dysregulation of the R/SI balance, offering a potential strategy for classifying patients based on immune status and matching them with specific therapies [273]. Although metagenomic next-generation sequencing can rapidly identify pathogens in patients with sepsis, its use in guiding antibiotic adjustment has not significantly improved patient prognosis. This suggests that pathogen identification should be better integrated with clinical diagnosis and treatment decision-making [274]. Results from a large statewide cohort study involving over 55,000 patients demonstrated that timely completion of the 3-hour sepsis bundle was associated with reduced risk-adjusted in-hospital mortality, with a more pronounced effect observed in the δ subtype [275]. These studies also highlight metabolic disruption of critical pathways during sepsis, which not only offers new targets for intervention but also promotes precision medicine in sepsis management through multidisciplinary approaches [276]. Studies of sepsis-associated acute kidney injury (SA-AKI) have identified microcirculatory dysfunction, inflammasome activation, and metabolic reprogramming as important pathogenic elements, thereby providing a basis for molecular target-based therapies [277].

In the chronic phase of sepsis, gut microbiota dysbiosis may persist for 14–21 days after onset. Notably, sex-specific differences have been observed, with females exhibiting greater

resilience than males. This observation may open up a unique point for microbiota-based intervention in sepsis [278]. Meanwhile, single-cell sequencing disclosed an atypical lymphocytic transcriptional signature in late sepsis characterized by the concomitance of immunoparalysis and micro-inflammation. This offers new possible applications for precision immunotherapy [279]. Early use of norepinephrine in septic shock can rapidly increase and stabilize arterial blood pressure, and may help limit prolonged hypotension and fluid overload. Observational studies, including propensity score-based analyses, have reported reduced fluid administration and inconsistent effects on day-28 mortality with early norepinephrine initiation, whereas a randomized controlled trial using fixed-dose norepinephrine (0.05 μg/kg/min) demonstrated improved shock control without a confirmed survival benefit, suggesting outcome heterogeneity across patient subgroups [280]. Detailed studies on the accurate classification of sepsis have shown that SR-BI deficiency is associated with sepsis of the relative adrenal insufficiency subtype, a chronic subgroup with sustained excessive inflammation. These studies also show that glucocorticoids are effective for this subtype, highlighting the importance of bundle therapy in sepsis [281]. Furthermore, the combination of IL-10 and the NEWS score provides important biomarker support for early sepsis stratification by identifying patients with low clinical scores and high physiological risk, offering a new avenue for clinical prognostic evaluation [204].

Unique medical approaches to sepsis are emerging through accurate immune profiling, metabolic monitoring, and mechanistic research, which facilitate early identification, risk stratification, and individualized therapy. Detailed characterization of host immune responses and metabolic disturbances provides new opportunities for precision treatment and improved patient outcomes. However, translating these advances into routine clinical practice remains challenging due to the complexity and heterogeneity of sepsis, which highlights the need for further investigation and clinical validation.

7 DISCUSSION

Despite substantial advances in sepsis research over the past decades, its clinical translation remains challenging. The pathophysiology of sepsis is highly complex, making it difficult for single-target therapies to maintain sustained therapeutic efficacy. One major reason is the dynamic biphasic nature of the immune response during sepsis. In the early hyperinflammatory phase, Tregs help limit excessive inflammation and reduce tissue damage. However, during the subsequent immunosuppressive phase, the expansion of Tregs may further suppress effector immune responses, thereby exacerbating immunosuppression and contributing to poor clinical outcomes [282]. Consequently, conventional anti-inflammatory therapies often overlook this stage-dependent immune regulation, making the timing, intensity, and duration of intervention critical determinants of therapeutic efficacy. For example, inhibitors targeting TLR4 demonstrated promising early efficacy in experimental models but failed to improve outcomes in clinical trials, highlighting the importance of appropriate therapeutic timing and immune status assessment. In addition, innate immune signaling pathways exhibit complex regulatory roles in sepsis. Although sepsis drives persistent platelet activation and dysfunction, TLR7 deficiency has been shown to preserve platelet function and modulate exosome-mediated platelet activation, suggesting that TLR7 may represent a key regulator of sepsis-associated platelet dysfunction and a potential therapeutic target [283].

Moreover, the marked heterogeneity of sepsis represents another major obstacle to precision medicine. Immune responses in sepsis are shaped by multiple endogenous and exogenous factors, including genetic background, age, sex, comorbidities, metabolic status, prior microbial exposure, and pharmacological interventions [284]. Multi-omics analysis indicates that immune-metabolic subtypes, including glycolysis-dominant and oxidative types, may underlie differential responses to treatment. Accordingly, future clinical studies should employ personalized interventions according to molecular subtypes, rather than a uniform strategy [285]. The interaction between inflammation and metabolic networks still requires more systematic analysis. Mitochondrial dysfunction, ferroptosis, and NLRP3 inflammasome activation represent common nodes in organ damage, but their timing and tissue specificity have yet to be fully elucidated [286]. By integrating metabolomics, spatial transcriptomics, and single-cell immune profiling, it is expected to uncover organ-specific pathological pathways, enabling more precise intervention design.

With respect to the treatment, several new immunomodulatory and antioxidant treatments have provided promising results. Targeting multiple aspects of these pathways has been observed to enhance treatment responsiveness in systemic models, leading to improved survival rates. Examples of such approaches include autophagy enhancement, Nrf2 activation, and pyroptosis inhibition (e.g., rapamycin, MitoTEMPO, and songorine), indicating the potential for multi-pronged therapeutic strategies [129]. Meanwhile, the application of exosomes from stem cells, non-coding RNAs, and gut microbiota regulation is uncovering new avenues for systemic immune reconstitution. When integrated with AI-predictive models and real-time biomarker feedback in the future, digital twin models may become increasingly feasible.

Interdisciplinary integration is likely to be a major direction in future sepsis research. The integration of immunology, metabolomics, systems biology and AI will enable a comprehensive understanding of the “pathogen-to-host network”. Through developing interpretable immune-metabolic-genetic multi-dimensional models, it will not only reveal the mechanisms underlying individual susceptibility but also guide clini-

cal practice toward precise, dynamic, and reproducible personalized interventions.

ABBREVIATIONS

8-OHdG, 8-Hydroxy-2'-deoxyguanosine; AKI, Acute kidney injury; AKK, Akkermansia muciniphila; ALI, Acute lung injury; Ang-2, Angiopoietin-2; AQP3, Aquaporin-3; ARDS, Acute Respiratory Distress Syndrome; BM, Bone marrow; C5ar1, C5a receptor 1; CETP, Cholesteryl Ester Transfer Protein; CLP, Cecal ligation and puncture; DNMT, DNA methyltransferase; EIF2AK2, Eukaryotic translation initiation factor 2α kinase 2; ERK, Extracellular Signal-Regulated Kinase; GA, Glatiramer acetate; GM-CSF, Granulocyte-Macrophage Colony-Stimulating Factor; GPR, G protein-coupled receptors; GPX4, Glutathione Peroxidase 4; GSDMD, Gasdermin D; H3K14la, Histone H3 lysine 14 lactylation; HDAC, Histone Deacetylase; HDL, High-Density Lipoprotein; HLA, Human Leukocyte Antigen; HLH, Hemophagocytic lymphohistiocytosis; HLJ1, Heat shock protein 40-like protein 1; HMGB1, High mobility group box 1; ICOS, Inducible T-cell Co-Stimulator; LNP, Lipid nanoparticles; LPIN1, Lipin 1; LPO, Lipid Peroxidation; LPS, Lipopolysaccharide; LqSOFA, Liverpool quick Sequential Organ Failure Assessment; m^6A, N6-methyladenosine; MALAT1, Metastasis-Associated Lung Adenocarcinoma Transcript 1; MCP-1, Monocyte Chemoattractant Protein-1; MDSCs, Myeloid-derived suppressor cells; METTL, Methyltransferase-Like; MHC, Major histocompatibility complex; MitoTEMPO, Mitochondria-targeted 2,2,6,6-tetramethylpiperidine-1-oxyl; MMP, Matrix metalloproteinase; MSCs, Mesenchymal stem cells; MyD88, Myeloid differentiation primary response 88; NETosis, Neutrophil Extracellular Trap formation; NETs, Neutrophil extracellular traps; NEWS, National Early Warning Score; NLRs, NOD-like receptors; NOP, Nociceptin/Orphanin FQ receptor; Nrf2, Nuclear factor erythroid 2–related factor 2; OLFM4, Olfactomedin 4; PAD4, Peptidylarginine Deiminase 4; PADI, Peptidyl Arginine Deiminase; PAI-1, Plasminogen Activator Inhibitor-1; PANX1, Pannexin 1; PGC-1α, Peroxisome Proliferator-Activated Receptor Gamma Coactivator-1 Alpha; PICS, Persistent Inflammation, Immunosuppression, and Catabolism Syndrome; PINK1, PTEN-induced kinase 1; PKM, Pyruvate Kinase, Muscle; PMN, Polymorphonuclear Myeloid; PRRs, Pattern recognition receptors; RANTES, Regulated upon Activation, Normal T Cell Expressed and Secreted; RKH, Tripeptide Arginine-Lysine-Histidine; ROCK, Rho-associated coiled-coil containing protein kinase; ROS, Reactive Oxygen Species; S1P, Sphingosine-1-phosphate; SAA, Serum amyloid A; SA-AKI, Sepsis-Associated acute kidney injury; SI-AKI, Sepsis-induced acute kidney injury; SAE, Sepsis-associated encephalopathy; Skp2, S-phase kinase-associated protein 2; SLC3A2, Solute Carrier Family 3 Member 2; SOFA, Sequential Organ Failure Assessment; SphK1, Sphingosine kinase 1; SRS, Sepsis Response Signature; SuPAR, Soluble urokinase-type plasminogen activator receptor; Tim-3, T-cell immunoglobulin and mucin domain-containing protein 3; TLRs, Toll-like receptors; TSLP, Thymic stromal lymphopoietin; VDAC2, Voltage-Dependent Anion Channel 2; VEGF, Vascular Endothelial Growth Factor; VISTA, V-domain Immunoglobulin Suppressor of T-cell Activation.

DECLARATIONS

Author contributions

Xuesong Liu, Jiejin Yang, and Zhihao Hu contributed equally to this work and share first authorship. Xuesong Liu, Jiejin Yang, and Zhihao Hu were responsible for conceptualization, literature investigation, and writing the original draft. Huiting Wei and Rong Zhang contributed to validation and formal analysis. Zhuoqun Huang performed visualization and prepared the figures and tables. Jiao Song supervised the project and provided critical revisions for important intellectual content, and served as the corresponding author. All authors have read and approved the final manuscript.

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Competing interests

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