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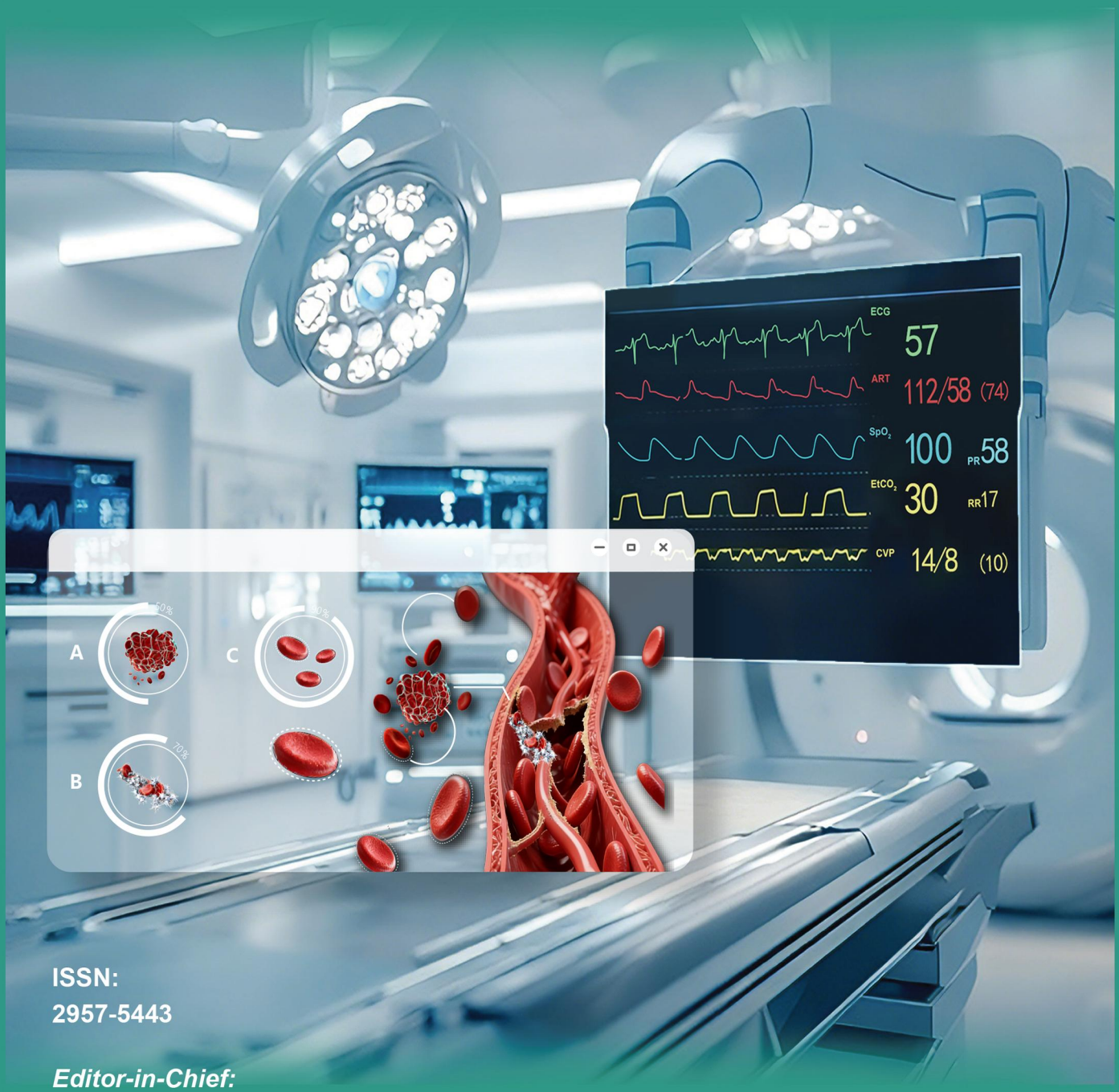
PERIOPERATIVE PRECISION MEDICINE

Volume 4 • Issue 1 • 2026



ISSN:
2957-5443

Editor-in-Chief:
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Perioperative
Precision Medicine

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REVIEW ARTICLE

Dysregulated cell death and inflammation in perioperative medicine: Mechanisms and therapeutic opportunities

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Received October 3, 2025; Accepted February 28, 2026; Published March 31, 2026

DOI: 10.61189/775612vwvmhm

Abstract

Cell death is a fundamental process influencing tissue homeostasis and recovery following surgical trauma. In the perioperative context, regulated cell death aids in clearing damaged cells and initiating repair. However, excessive or dysregulated cell death—often exacerbated by ischemia-reperfusion injury, surgical stress, and anesthetic agents—can trigger intense inflammatory responses, leading to complications such as postoperative organ dysfunction (e.g., acute lung injury, delirium, and myocardial injury), impaired wound healing, and increased risk of infections. Mitochondrial dysfunction, a key contributor to these processes, releases damage-associated molecular patterns, further amplifying perioperative inflammation. Emerging evidence suggests that modulating specific cell death pathways (e.g., apoptosis, pyroptosis, necroptosis) and associated inflammatory signaling (e.g., via NLRP3 inflammasome or TLR4 inhibition) may offer novel strategies to improve perioperative outcomes. This review explores the interplay between cell death and inflammation, with emphasis on perioperative relevance, and discusses emerging therapeutic targets that may facilitate precision medicine in surgical patients.

Keywords: Cell death, Perioperative medicine, Inflammation, Ischemia-reperfusion injury, Damage-associated molecular patterns, Precision medicine, Organ protection

1 INTRODUCTION

The interaction network of cell death and inflammation is an important and rapidly developing field in current biomedical research. This interplay not only plays a crucial role in maintaining tissue homeostasis but also is central to the pathogenesis and progression of multiple diseases [1]. It represents a critical determinant of patient outcomes in the perioperative period. Surgical trauma, anesthesia, ischemia-reperfusion injury, and blood loss can disrupt cellular homeostasis, triggering programmed cell death pathways and robust inflammatory responses. While these processes are essential for tissue repair and defense, their dysregulation contributes significantly to postoperative complications, including acute kidney injury, acute respiratory distress syndrome, sepsis, postoperative cognitive dysfunction (POCD), and impaired wound healing. Cell death and inflammation are key physiological processes by which organisms respond to internal and external stimuli, and the complex relationship between the two has profound implications for both health and disease. Cell death, once simply

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considered a way for organisms to clear useless or damaged cells, is now understood to be a highly regulated process that includes various distinct forms, such as apoptosis, necroptosis, pyroptosis, ferroptosis, and cuproptosis [2]. These modes of cell death not only differ in morphological and biochemical characteristics, but also play different roles in the regulation of inflammatory reactions [3]. For example, apoptosis is generally considered immunologically silent, and can be removed by efficient efferocytosis without triggering an inflammatory reaction. However, apoptotic cells can also release damage-associated molecular patterns (DAMPs), thereby activating immune responses. Studies have shown that cell death can affect inflammatory reactions through various mechanisms [4, 5]. On the one hand, DAMPs released by cell death can activate pattern recognition receptors (PRRs), thereby initiating inflammatory signaling pathways and promoting the production and release of inflammatory cytokines. On the other hand, inflammatory cytokines, such as TNF-α and interferons, can directly induce cell death, forming a positive feedback loop that exacerbates inflammatory reactions and tissue injury. Cell death can be both a trigger for inflammation and a result of inflammatory reactions, and this dual role leads to the complexity of interaction networks.

In perioperative medicine, the balance between beneficial and detrimental cell death is delicate. Apoptosis, for instance, typically resolves without inflammation, but impaired clearance of apoptotic cells can lead to secondary necrosis and DAMP release. Conversely, highly inflammatory death pathways like pyroptosis and necroptosis are increasingly recognized as key drivers of organ injury following surgical stress. Mitochondria, central to cellular energetics, also act as crucial signaling hubs; perioperative insults (e.g., hypoxia, oxidative stress) can cause mitochondrial dysfunction, promoting DAMP release (e.g., mtDNA, ATP) and activating innate immune receptors (e.g., TLR9, NLRP3). This initiates a cascade of pro-inflammatory cytokine production (e.g., IL-1β, IL-18, TNF-α), leading to further tissue damage and a potential cycle of inflammation and cell death.

Although there is a preliminary understanding of the interaction between cell death and inflammation, the specific molecular mechanisms and signaling networks remain incompletely understood. In particular, current research still lacks systematic integration regarding how various forms of cell death synergistically affect inflammatory reactions, the specific mechanisms of action of cell death and inflammation in different diseases, and how these networks temporally and spatially regulate the onset and pathological progression of inflammation [6]. Exploring the contribution of different cell death mechanisms to inflammatory regulatory networks and their potential targets will help to more comprehensively understand inflammation-related diseases and provide a theoretical basis for developing new therapeutic methods. Simultaneously, understanding the specific roles of different cell death modes and their complex crosstalk with inflammatory signals in the perioperative context is crucial. This knowledge paves the way for novel therapeutic strategies aimed at modulating these pathways to protect vulnerable organs, mitigate excessive inflammation, and promote recovery.

This review systematically analyzes cell death (apoptosis, pyroptosis, necroptosis, etc.) and the dynamic interaction mechanisms of inflammatory reactions, focusing on the spatiotemporal patterns of specific DAMPs (e.g., High Mobility Group Box 1 [HMGB1], mtDNA) that are released by different death pathways and that activate PRRs (TLR4/NLRP3), and elucidating the signal transduction logic of the "DAMPs-PRRs-inflammatory cascade". For sepsis, autoimmune diseases, and metabolic diseases, it deeply analyzes the cascade effects of key pathway imbalances such as NETosis triggering type I interferon storms, GSDMD-mediated IL-1β self-amplification cycles, and lipotoxicity-induced death mode switching, combining spatial transcriptomics and single-cell metabolomics technologies to reveal organ microenvironment specificity. Based on this, this review critically evaluates the Janus-faced nature of targeted intervention strategies (such as RIPK1 inhibitors), explores the mechanisms by which cell death and inflammation lead to perioperative pathology, emphasizes their clinical relevance, and validates their therapeutic potential in alleviating sepsis-induced multiple organ failure and chronicization of autoimmune diseases.

2 MOLECULAR MECHANISMS OF CELL DEATH

Since the phenomenon of cell death was first described in the 19th century, the field has undergone a profound transformation from morphological observation to molecular decoding, as shown in **Figure 1**. Early on, Kerr et al. bifurcated the modes of death into apoptosis and necrosis based on microscopic features. With the discovery of gene regulatory pathways, the concept of programmed cell death (PCD) emerged [7]. Over the last decade, breakthroughs in high-throughput technologies have further revealed metabolism-associated cell death such as ferroptosis and cuproptosis, as well as integrative cell death programs such as PANoptosis [8]. Today's cell death classification system has shifted from a single morphological criterion to multidimensional definitions based on molecular execution mechanisms, regulatory characteristics, and immunological outcomes, as shown in **Table 1**. This section will systematically analyze eight core death modalities, ranging from classic apoptosis and pyroptosis to emerging cuproptosis and PANoptosis, focusing on their molecular switches, morphological hallmarks, and association with inflammation, thereby laying the foundation for understanding the interaction between death and inflammation.

2.1 Apoptosis

Apoptosis is a form of programmed cell death strictly regulated by genes, and is essential for the growth and development, tis-

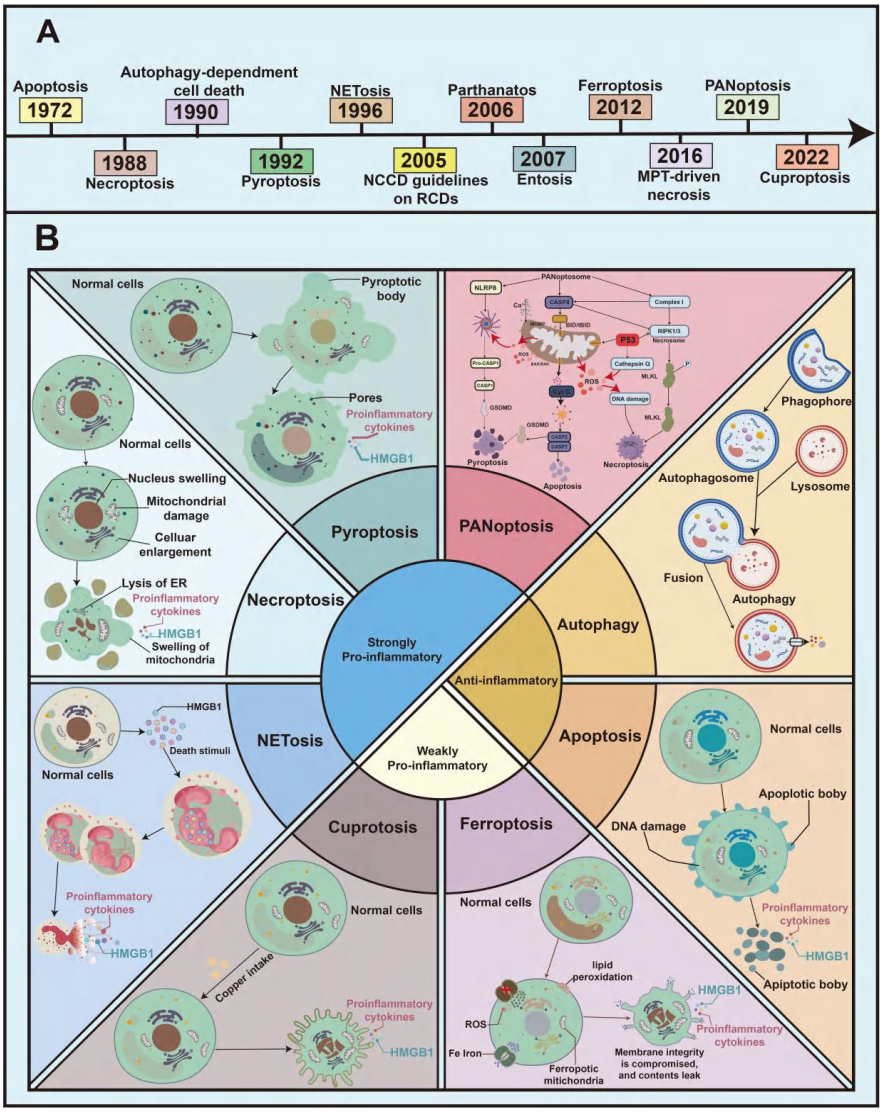

Figure 1. Historical evolution and mechanistic landscape of regulated cell death pathways. (A) Timeline of key discoveries in the field of RCD from 1972 to 2022. Major RCD modalities are annotated with their year of nomenclature or seminal characterization, illustrating the rapid expansion of our understanding beyond apoptosis. (B) A comprehensive schematic diagram categorizing distinct RCD pathways based on their inflammatory potential (ranging from strongly pro-inflammatory to weakly pro-inflammatory or anti-inflammatory) and molecular mechanisms. The central area highlights key pro-inflammatory pathways (necroptosis, pyroptosis, PANoptosis), while the periphery details other modalities. For each pathway, representative cellular and subcellular events are depicted, including morphological changes (e.g., pyknotic nuclei in apoptosis, pore formation in pyroptosis), organelle dynamics (e.g., mitochondrial damage in ferroptosis), and the release of key mediators (e.g., HMGB1, proinflammatory cytokines). This integrative overview emphasizes the connection between specific molecular effectors and the resultant immunological outcomes of different cell death types. RCD, regulated cell death; HMGB1, High Mobility Group Box 1; NETosis, NET formation cell death; NCCD, Nomenclature Committee on Cell Death; MPT, mitochondrial permeability transition; ER, endoplasmic reticulum; NLRP8, NLR family pyrin domain containing 8; CASP8, caspase-8; CASP1, caspase-1; GSDMD, gasdermin D; MOMP, mitochondrial outer membrane permeabilization; BID, BH3-interacting domain death agonist; tBID, truncated BID; ROS, reactive oxygen species; BAX, BCL2-associated X protein; BAK, BCL2-antagonist/killer 1; Cyt C, cytochrome c; CASP3, caspase-3; CASP7, caspase-7; RIPK1/3, receptor-interacting protein kinase 1/3; P53, tumor protein p53; MLKL, mixed lineage kinase domain-like protein.

sue homeostasis, and immune function of multicellular organisms. Unlike necrosis, apoptosis is an active, ordered form of cell suicide [28]. The morphological hallmarks of apoptosis primarily include cell shrinkage, chromatin condensation, and the formation of apoptotic bodies. Apoptotic cells decrease in volume, their cytoskeleton disintegrates, and they detach from surrounding cells. The nucleus undergoes significant changes, with chromatin condensing and aggregating at the nuclear periphery, followed by DNA fragmentation, forming nuclear fragments of varying sizes. The cell membrane blebs to form apoptotic bodies. These bodies, which contain organelles and cytoplasm, are eventually phagocytosed and cleared by neighboring cells or macrophages without eliciting an inflammatory response [29].

Apoptosis is regulated by various factors, including intracellular and extracellular signals, as shown in **Figure 2**. Intracellular signaling pathways primarily involve the mitochondrial pathway, in which BCL-2 family proteins (e.g., Bax, Bak) play a crucial role in regulating mitochondrial membrane permeability. Increased mitochondrial membrane permeability leads to the release of cytochrome c, activating the caspase protease family, and ultimately leading to apoptosis [30]. Extracellular signaling pathways, on the other hand, are mediated by death receptors. For example, Fas ligand binds to the Fas receptor, activating caspase-8, which then initiates downstream apoptotic processes [31]. In addition to the classical apoptotic pathways, there also exist caspase-independent apoptotic pathways. For example, the release of apoptosis-inducing factor and endonuclease G can also lead to chromatin condensation and DNA fragmentation, thereby causing cell death [32].

The mitochondrial (intrinsic) pathway of apoptosis is tightly regulated by BCL-2 family proteins, which are mainly divided into two categories: pro-apoptotic and anti-apoptotic. Pro-apoptotic proteins include Bax and Bak, which, upon receiving apoptotic signals, undergo conformational changes and oligomerization, ultimately leading to mitochondrial outer membrane permeabilization (MOMP) [33]. The occurrence of MOMP is a critical step in the intrinsic apoptotic path-

Table 1. Core molecular characteristics of cell death modalities and their association with inflammation

Cell death modality	Subtype/key pathway	Core molecular executors	Triggering signals	Morphological/ biochemical features	Key regulatory nodes	Inflammatory intensity	Key DAMPs released	References
Apoptosis	Mitochondrial pathway	Cytochrome c, Apaf-1, Caspase-9	DNA damage, ROS	Cell shrinkage, chromatin condensation	Bcl-2/Bax balance	Low	Phosphatidylserine, nucleosomes	[9]
	Death receptor pathway	Caspase-8, FADD	TNF-α, FasL	Membrane blebbing, apoptotic bodies	c-FLIP regulation		/	[10]
	ER stress pathway	Caspase-12, CHOP	ER stress	Cytoplasmic vacuolization	IRE1α-XBP1 axis		ATP	[11]
Pyroptosis	Canonical inflammasome	NLRP3/ASC/ Caspase-1, GSDMD	ATP, K^+ efflux	Cell swelling, GSDMD pore formation	NEK7, ASC oligomerization	High	IL-1β, IL-18, HMGB1	[12]
	Non-canonical pathway	Caspase-4/5/11, GSDMD	Intracellular LPS	Membrane rupture, LDH release	Guanylate-binding proteins		mtDNA	[13]
	Caspase-8-dependent	Caspase-8, GSDMC/E	TNF-α, TLR2/4	Hybrid pyroptotic-apoptotic features	cFLIP regulation		S100A8/9	[14]
Necroptosis	RIPK1-dependent	RIPK1, RIPK3, MLKL	TNF-α (during caspase inhibition)	Membrane permeabilization, cell lysis	RIPK1 ubiquitination status	High	HMGB1, mtDNA	[15]
	RIPK1-independent	ZBP1, RIPK3, MLKL	Viral RNA, IFNs	RIPK3 phosphoaggregation	Zα-domain activation		Heat shock proteins	[16]
Ferroptosis	GPX4-inhibition pathway	GPX4, ACSL4, LPCAT3	Iron overload, Erastin/RSL3	Mitochondrial shrinkage, lipid peroxidation	NRF2-Keap1 axis	Moderate	4-HNE, MDA	[17]
	System Xc^- inhibition	SLC7A11↓, GSH depletion	Cystine deprivation, sorafenib	Lipid ROS accumulation	FSP1 membrane localization		Oxidized phospholipids	[18]
Cuproptosis	Mitochondrial proteotoxicity	FDX1, DLAT, LIAS	Copper ion overload	Mitochondrial protein oligomerization	Copper chaperones (ATOX1)	Moderate	mtDNA, mtROS	[19]
NETosis	Suicidal NETosis	PAD4, MPO, NE	Pathogens, immune complexes	NETs release (chromatin decondensation)	ROS-Ca^{2+} axis	Extreme	Citrullinated histones, LL-37	[20]
	Vital NETosis	/	Cytokine storm	Preserved nuclear envelope	SK3 channel activation		Antimicrobial peptides	[21]
Autophagic cell death	Atg5/7-dependent	Beclin-1, LC3-II	Starvation, rapamycin	Autolysosomal vacuolation	mTOR-ULK1 axis	Low/ Moderate	Low ATP	[22]
	Rab9-dependent alternative	Rab9, UVRAG	Stress-induced	LC3-unlipidated vacuoles	TRAPPIII complex		/	[23]
Alkaliptosis	Na^+/H^+ exchanger dysregulation	NHE1, ASIC1a	Intracellular alkalosis	Cell swelling, vacuolation	Lysosomal pH homeostasis	Low	Cathepsins	[24]
Lysosome-dependent cell death	Cathepsin leakage	Cathepsin B/D, LAMP1	Lysosomal membrane permeabilization	Lysosomal rupture	Galectin-3 surveillance	Moderate	Lysosomal enzymes	[25]
PANoptosis	ZBP1-PANoptosome	ZBP1, RIPK1, Caspase-8	Viral RNA, cytokine storm	Hybrid death features	IRF1 transcriptional regulation	Extreme	Cooperative DAMPs release	[26]
	AIM2-PANoptosome	AIM2, ASC, FADD	Cytosolic DNA	PANoptosome oligomerization	cGAS-STING interaction		/	[27]

Note: DAMPs, damage-associated molecular patterns; Apaf-1, apoptotic protease activating factor 1; ROS, reactive oxygen species; Bcl-2, B-cell lymphoma 2; Bax, BCL2-associated X protein; FADD, Fas-associated death domain protein; TNF-α, tumor necrosis factor-alpha; FasL, Fas ligand; c-FLIP, cellular FLICE (FADD-like IL-1β-converting enzyme)-inhibitory protein; ER, endoplasmic reticulum; CHOP, C/EBP homologous protein; IRE1α, inositol-requiring enzyme 1 alpha; XBP1, X-box binding protein 1; ATP, adenosine triphosphate; NLRP3, NLR family pyrin domain containing 3; ASC, apoptosis-associated speck-like protein containing a CARD; GSDMD, gasdermin D; NEK7, NIMA-related kinase 7; IL-1β, interleukin-1β; IL-18, interleukin-18; HMGB1, high mobility group box 1; LPS, lipopolysaccharide; LDH, lactate dehydrogenase; mtDNA, mitochondrial DNA; GSDMC, gasdermin C; GSDME, gasdermin E; TLR2, Toll-like receptor 2; TLR4, Toll-like receptor 4; S100A8/9, S100 calcium-binding protein A8/A9; RIPK1, receptor-interacting protein kinase 1; RIPK3, receptor-interacting protein kinase 3; MLKL, mixed lineage kinase domain-like pseudokinase; ZBP1, Z-DNA-binding protein 1; IFNs, interferons; GPX4, glutathione peroxidase 4; ACSL4, acyl-CoA synthetase long-chain family member 4; LPCAT3, lysophosphatidylcholine acyltransferase 3; Erastin/RSL3, ferroptosis inducers (Erastin and RAS-selective lethal 3); NRF2, nuclear factor erythroid 2-related factor 2; Keap1, Kelch-like ECH-associated protein 1; 4-HNE, 4-hydroxynonenal; MDA, malondialdehyde; System Xc^-, cystine/glutamate antiporter; SLC7A11, solute carrier family 7 member 11; GSH, glutathione; FSP1, ferroptosis suppressor protein 1; FDX1, ferredoxin 1; DLAT, dihydrolipoamide S-acetyltransferase; LIAS, lipoic acid synthetase; ATOX1, antioxidant 1 copper chaperone; mtROS, mitochondrial reactive oxygen species; PAD4, peptidylarginine deiminase 4; MPO, myeloperoxidase; NE, neutrophil elastase; NETs, neutrophil extracellular traps; Ca^{2+}, calcium ion; SK3, small conductance calcium-activated potassium channel 3; LL-37, cathelicidin antimicrobial peptide; Atg5, autophagy-related protein 5; Atg7, autophagy-related protein 7; LC3-II, microtubule-associated protein 1 light chain 3-II; mTOR, mechanistic target of rapamycin; ULK1, UNC-51-like kinase 1; Rab9, Ras-related protein Rab-9; UVRAG, UV radiation resistance-associated gene protein; TRAPPIII, transport protein particle III complex; NHE1, Na^+/H^+ exchanger 1; ASIC1a, acid-sensing ion channel 1a; Cathepsins, lysosomal proteases; LAMP1, lysosomal-associated membrane protein 1; Galectin-3, β-galactoside-binding lectin 3; IRF1, interferon regulatory factor 1; AIM2, absent in melanoma 2; cGAS, cyclic GMP-AMP synthase; STING, stimulator of interferon genes.

way, as it leads to the release of cytochrome c from the mitochondrial intermembrane space into the cytoplasm. In the cytoplasm, cytochrome c binds to Apaf-1 and recruits pro-caspase-9 to form the apoptosome [34]. The apoptosome activates caspase-9, and activated caspase-9 subsequently activates downstream effector caspases, such as caspase-3 and caspase-7. These effector caspases are responsible for cleaving various intracellular substrates, ultimately leading to apoptosis [35]. Anti-apoptotic proteins, such as BCL-2 and BCL-xL, inhibit apoptosis by binding to Bax and Bak, preventing their oligomerization and MOMP [36]. BH3 domain proteins (e.g., Bid, Bim, Puma, Noxa, and Bad) initiate apoptosis by binding to anti-apoptotic proteins. Cellular stress and damage activate BH3 proteins, which can bind to anti-apoptotic BCL-2 proteins, enabling Bax and Bak to oligomerize and induce MOMP [37]. The balance between pro-apoptotic and anti-apoptotic proteins determines cell fate. In the extrinsic pathway, cell surface death receptors, such as Fas receptor and TNFR1, initiate apoptotic signals after binding to their respective ligands (FasL and TNF-α) [38]. After FasL binds to the Fas receptor, the Death-Inducing Signaling Complex is formed, which contains FADD and caspase-8. In the complex, caspase-8 is activated, and activated caspase-8 can directly activate downstream caspase-3, initiating the execution of apoptosis. Additionally, caspase-8 can cleave the cytoplasmic Bid protein, producing truncated Bid (tBID). tBID localizes to mitochondria, inducing the release of cytochrome c, thereby cross-linking with the endogenous pathway and amplifying the apoptotic signal [39]. After TNF-α binds to TNFR1, this interaction recruits proteins such as TRADD, FADD, and RIP to form Complex I, activating the NF-κB signaling pathway, leading to anti-apoptotic effects. However, TNFR1 can also indirectly induce apoptosis through FADD-dependent apoptosis and caspase-8, and activated caspase-8 can further activate other apoptotic enzymes such as caspase-3 [35]. Activation of the extrinsic pathway depends on the expression levels and functional status of death receptors, adaptor proteins, and caspases. The two main apoptotic pathways do not operate independently but rather interact with and regulate each other during apoptosis. For example, in the extrinsic pathway, caspase-8 activates Bid, generating tBID, thereby activating the mitochondrial pathway [10]. In the intrinsic pathway, cytochrome c released by mitochondria activates caspase-9, and activated caspase-9 may in turn affect certain components of the death receptor pathway [40]. This crosstalk between pathways ensures the precise regulation and efficient execution of the apoptotic process. Furthermore, BCL-2 family proteins not only regulate the permeability of the mitochondrial outer membrane but also participate in the regulation of endoplasmic reticulum and calcium ion signaling, further influencing cellular apoptotic sensitivity [41, 42].

There is a complex bidirectional relationship between apoptosis and inflammation, where apoptosis can both trigger and inhibit the occurrence of inflammatory responses [43]. Apoptosis is generally considered an immunologically silent mode of cell death that can prevent the occurrence of inflammation. However, in certain circumstances, the apoptotic process itself may trigger inflammatory responses [44]. For example, if apoptotic cells are not promptly cleared, they may undergo secondary necrosis, releasing cellular contents, thereby activating the immune system and inducing inflammation and autoimmune responses. Furthermore, apoptotic cells can also release some endogenous DAMPs, which activate immune cells and promote inflammatory responses [45]. On the other hand, inflammatory responses can also affect the occurrence of apoptosis through multiple pathways. Pro-inflammatory cytokines in the inflammatory environment, such as TNF, can directly induce apoptosis [46]. In addition, substances such as

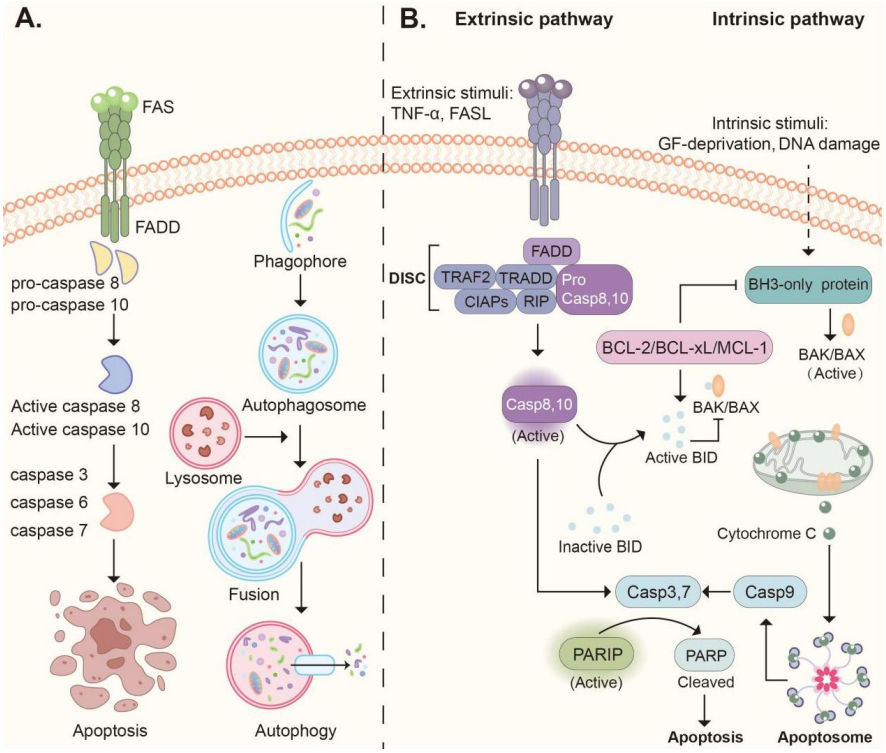

Figure 2. Molecular mechanisms of the extrinsic and intrinsic apoptotic pathways. (A) Schematic overview of a cell, contextualizing the apoptotic signaling cascades within the cellular architecture. (B) Detailed schematic diagram illustrating the key signaling cascades of apoptosis. The extrinsic (death receptor) pathway (left panel) is initiated by the binding of extracellular ligands (e.g., FASL, TNF-α) to their respective death receptors (e.g., FAS). This leads to the formation of the DISC, which recruits and activates initiator Casp8 and Casp10. The intrinsic (mitochondrial) pathway (right panel) is activated by intracellular stresses such as growth factor deprivation or DNA damage, leading to the activation of pro-apoptotic BCL-2 family proteins (e.g., BID, BAX, BAK). This results in MOMP and the release of Cyt C. Cyt C, together with Apaf-1, forms the apoptosome, activating Casp9. Both pathways converge on the activation of executioner Casp3 and Casp7, which cleave cellular substrates like PARP, ultimately leading to apoptotic cell death. Notably, crosstalk between the pathways occurs via Casp8-mediated cleavage of BID to tBID, which amplifies the apoptotic signal through the mitochondrial pathway. FADD, Fas-associated death domain protein; TNF-α, tumor necrosis factor-alpha; FASL, Fas ligand; TRAF2, TNF receptor-associated factor 2; TRADD, TNF receptor-associated death domain protein; CIAPs, cellular inhibitors of apoptosis proteins; RIP, receptor-interacting protein; Casp8, caspase-8; Casp9, caspase-9; Casp10, caspase-10; BID, BH3-interacting domain death agonist; BAK, BCL2-antagonist/killer 1; BAX, BCL2-associated X protein; Casp3, caspase-3; Casp7, caspase-7; PARP, poly(ADP-ribose) polymerase; Apaf-1, apoptotic protease activating factor 1; tBID, truncated BID; MOMP, mitochondrial outer membrane permeabilization; Cyt C, cytochrome c; FAS, Fas cell surface death receptor; DISC, Death-Inducing Signaling Complex; GF-deprivation, growth factor deprivation; BCL-2, B-cell lymphoma 2; BCL-xL, B-cell lymphoma-extra large; MCL-1, Myeloid cell leukemia 1; PARP, Poly(ADP-ribose) polymerase.

reactive oxygen species (ROS) and reactive nitrogen species produced during the inflammatory response may also damage cells and promote the occurrence of apoptosis [47]. Therefore, apoptosis and inflammation mutually influence each other, jointly participating in maintaining body homeostasis. The occurrence of inflammation is closely related to the life cycle of immune cells. Apoptosis, as a form of programmed cell death, plays an important role in the process of inflammation resolution. By promoting the apoptosis of inflammatory cells such as neutrophils, the intensity and duration of the inflammatory response can be effectively controlled [48]. Studies have shown that the pro-apoptotic protein ARTS can induce neutrophil apoptosis, promote the clearance of apoptotic cells, and remodel macrophage function, thereby promoting inflammation resolution [49]. During the clearance of apoptotic cells, macrophages release anti-inflammatory factors, further inhibiting the inflammatory response [50]. Zinc has antioxidant, anti-inflammatory, and cell death-regulating effects [51]. It can inhibit the activity of caspase-3, -7, and -8, thereby regulating caspase-dependent apoptosis and necroptosis. In diseases such as COVID-19 and sepsis, the immunomodulatory role of zinc has received widespread attention.

2.2 Pyroptosis

Pyroptosis, as an inflammatory form of programmed cell death, has a core molecular execution mechanism that primarily relies on the activation of Gasdermin family proteins, particularly GSDMD, and its process of forming pores on the cell membrane [52]. Pyroptosis not only plays a role in anti-infection immunity, protecting the body by clearing intracellular pathogens and damaged cells, but also plays an important role in tumor immunity, influencing the occurrence, development, and treatment of tumors. However, uncontrolled pyroptosis can lead to excessive inflammatory activation and tissue damage, and is closely related to the occurrence and development of various diseases, including cardiovascular diseases, neurological diseases, and autoimmune diseases [53]. Therefore, in-depth research into the molecular mechanisms and biological functions of pyroptosis helps to develop therapeutic strategies for related diseases.

The initiation of the classical pyroptosis pathway depends on the activation of intracellular inflammasomes, as shown in **Figure 3**. Inflammasomes are a type of multiprotein complex that recognizes pathogen-associated molecular patterns (PAMPs) and DAMPs. Common inflammasomes include NLRP3, NLRC4, NLRP1b, and AIM2, among others [54].

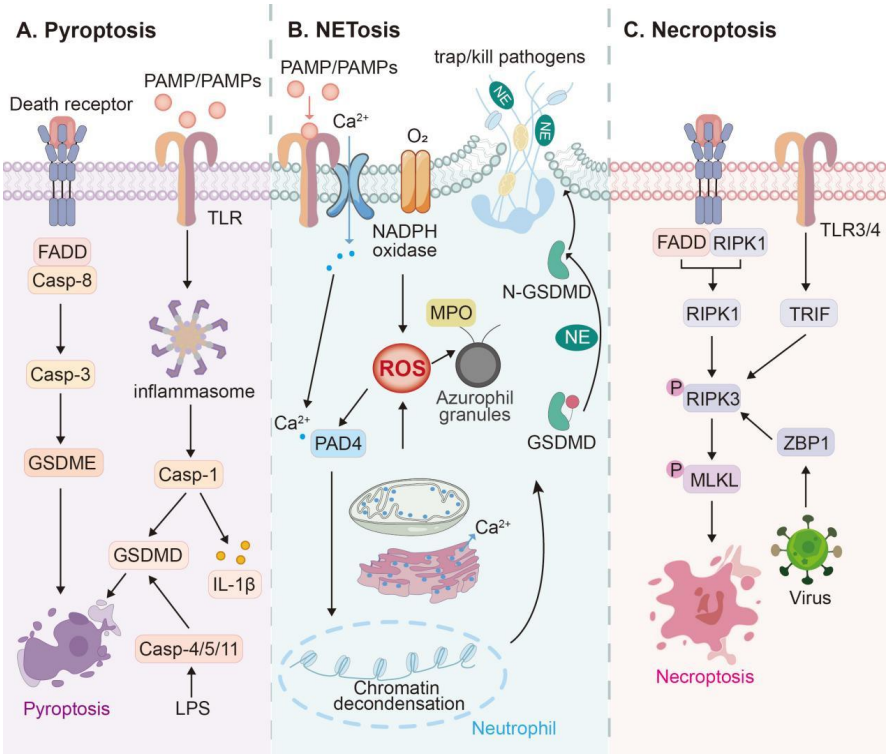

Figure 3. Molecular mechanisms of key pro-inflammatory cell death pathways: pyroptosis, NETosis, and necroptosis. Schematic models depicting the signaling cascades of three distinct pro-inflammatory regulated cell death pathways. (A) Pyroptosis. Activation by PAMPs or DAMPs through TLRs or death receptors recruits adaptor proteins (e.g., FADD) and activates inflammatory caspases (Casp-1/4/5/11) or, in certain contexts, Casp-8. These caspases cleave GSDMD or GSDME, liberating the N-GSDMD that oligomerizes and forms pores in the plasma membrane. This leads to osmotic lysis (pyroptosis) and the release of mature IL-1β. (B) NETosis. In neutrophils, PAMPs trigger Ca^{2+} influx and activate NADPH oxidase to generate ROS. ROS promote PAD4-mediated histone citrullination and chromatin decondensation. NE and MPO translocate to the nucleus, and together with GSDMD (which may facilitate the process), lead to the expulsion of NETs composed of DNA and granular proteins to trap pathogens. (C) Necroptosis. When triggered by viral infection or TNF signaling, necroptosis involves the inhibition of Casp-8 activity, which allows the activation of RIPK1 and RIPK3. RIPK3 phosphorylates MLKL, which oligomerizes, translocates to, and disrupts the plasma membrane, resulting in necroptotic cell death. These pathways represent crucial mechanisms by which the innate immune system eliminates infected or damaged cells while amplifying inflammatory responses. PAMP, pathogen-associated molecular pattern; TLR, Toll-like receptor; FADD, Fas-associated death domain protein; Casp-1, caspase-1; Casp-8, caspase-8; Casp-3, caspase-3; GSDME, gasdermin E; GSDMD, gasdermin D; IL-1β, interleukin-1β; Casp-4/5/11, caspase-4/5/11; LPS, lipopolysaccharide; NADPH oxidase, nicotinamide adenine dinucleotide phosphate oxidase; ROS, reactive oxygen species; PAD4, peptidylarginine deiminase 4; N-GSDMD, N-terminal fragment of gasdermin D; MPO, myeloperoxidase; TRIF, TIR-domain-containing adapter-inducing interferon-β; ZBP1, Z-DNA-binding protein 1; MLKL, mixed lineage kinase domain-like pseudokinase; RIPK1, receptor-interacting protein kinase 1; RIPK3, receptor-interacting protein kinase 3; NE, neutrophil elastase; TLR3/4, Toll-like receptor 3/4; DAMP, damage-associated molecular pattern; NET, neutrophil extracellular trap.

Upon activation, they recruit ASC and pro-caspase-1 to form an inflammasome complex. Within the complex, pro-caspase-1 is activated into caspase-1, which is capable of cleaving pro-IL-1β and pro-IL-18, converting them into mature IL-1β and IL-18. Simultaneously, caspase-1 also cleaves GSDMD, releasing its N-terminal domain (GSDMD-N) [55]. After oligomerization, GSDMD-N inserts into the cell membrane, forming pores, an event that leads to an imbalance of ion concentrations inside and outside the cell, cell swelling and rupture, and the release of cellular contents, including pro-inflammatory cytokines such as IL-1β and IL-18. The release of these cytokines further exacerbates the inflammatory response [56, 57]. The non-canonical pyroptosis pathway does not depend on caspase-1, but is activated through caspase-4/5/11 (caspase-11 in mice). These caspases can directly recognize intracellular LPS, thereby activating GSDMD [58]. Activated GSDMD-N also forms pores on the cell membrane, leading to pyroptosis and the release of inflammatory mediators. In addition to the classical and non-classical pathways, some other pathways are also involved in the regulation of pyroptosis. For example, caspase-8 can be activated through the TLR2/4 or TNFR signaling pathways, subsequently activating GSDMC/E. The expression of GSDME is suppressed in many tumor cells, but when its expression is restored, the activation of caspase-3 can lead to GSDME-mediated pyroptosis, thereby inhibiting tumor growth [10, 59]. Furthermore, mechanical force activates TRPV4, leading to calcium ion influx and stimulating the production of ROS, which can also ultimately lead to pyroptosis [60]. In-depth study of these pathways helps us better understand the essence of inflammatory responses and cell death, and provides new insights and strategies for the treatment of related diseases.

A significant context in which pyroptosis plays a critical pathogenic role is acute lung injury (ALI) during the perioperative period. Here, alveolar epithelial cell pyroptosis, driven by NLRP3 inflammasome activation, is a key mechanism amplifying injury. Surgical trauma, mechanical ventilation, hypoxia, or infections can provide both priming (e.g., via NF-κB upregulation of NLRP3 and pro-IL-1β) and activation signals (e.g., via K^+ efflux, ROS, or DAMPs) for the NLRP3 inflammasome [61]. Its assembly activates caspase-1, which cleaves GSDMD to cause pyroptotic pore formation, and processes pro-IL-1β into its mature, releasable form. This results in alveolar barrier disruption, edema, and a

potent local and systemic inflammatory cascade. Preclinical models, such as ventilator-induced lung injury, have been instrumental in delineating this pathway. Mechanical stress can activate NLRP3, and inhibition of this pathway (e.g., via FGF21 or ASK1 deficiency) attenuates ventilator-induced lung injury [62, 63]. Furthermore, in sepsis-induced ALI or one-lung ventilation settings, modulating the NLRP3/GSDMD axis (e.g., with sevoflurane, melatonin, or Rebastinib) shows protective effects, highlighting its potential as a therapeutic target to mitigate postoperative ALI [64-66].

Pyroptosis is an inflammatory form of cell death that amplifies inflammatory responses through multiple pathways and plays a crucial role in the pathophysiological processes of various diseases. Pyroptosis not only directly leads to the release of cellular contents but also indirectly amplifies inflammatory signals by activating multiple inflammatory pathways and immune cells, forming a complex inflammatory amplification network. After pyroptotic cells rupture, a large number of pro-inflammatory factors, such as IL-1β and IL-18, are released, which can significantly activate adjacent immune cells and tissue cells, further amplifying the inflammatory response [67, 68]. Among these, IL-1β is particularly crucial, as it can induce various cells to produce more inflammatory mediators (such as TNF-α and IL-6), forming an inflammatory cascade [69]. Secondly, the cellular contents and pro-inflammatory factors released by pyroptosis can strongly attract and activate immune cells such as neutrophils, macrophages, and T cells. These immune cells recruited to the inflammatory site will release more inflammatory mediators and ROS, exacerbating local tissue damage [70]. In addition, the pyroptosis-mediated inflammatory response profoundly affects the composition and structure of the extracellular matrix (ECM). For example, the upregulation of the ECM component Versican by cytokines in the early stage of inflammation promotes the progression of the inflammatory response [71]. At the same time, pyroptosis is closely linked to immunometabolism. Inflammatory signals can alter cellular metabolic pathways, while the metabolic state of immune cells (e.g., ATP metabolism) directly affects inflammasome activation and inflammatory factor release [72, 73]. For example, B cell-derived extracellular vesicles can affect $CD8^+$ T cell responses by hydrolyzing ATP. Under specific pathological conditions, such as systemic lupus erythematosus (SLE), autoantibodies (e.g., anti-dsDNA antibodies) can also amplify pyroptosis-related inflammatory responses.

This potent inflammatory amplification effect enables pyroptosis to play important roles in both host immune defense and disease development. Pyroptosis is a crucial mechanism for the host to combat pathogen infection. When pyroptosis is induced in infected cells, intracellular pathogens can be cleared, and inflammatory factors are released, activating immune cells and enhancing host immune responses [74]. In sepsis, although pyroptosis acts as a host defense mechanism to clear pathogens, the inflammatory storm caused by its overactivation can significantly worsen the condition [75]. Pyroptosis plays a complex role in tumor initiation and progression. On the one hand, pyroptosis can inhibit tumor initiation. Inducing pyroptosis in tumor cells can directly kill them, release tumor-associated antigens, and activate anti-tumor immune responses [76]. On the other hand, studies have shown that pyroptosis may also promote tumor metastasis and drug resistance [77]. Inflammatory factors released by pyroptosis can promote the formation of the tumor microenvironment and inhibit the activity of anti-tumor immune cells. Therefore, a deeper understanding of the regulatory mechanisms of pyroptosis and its role in diseases helps develop new therapeutic strategies to control inflammatory responses and improve disease prognosis. For example, regulating the ubiquitination level of the NLRP3 inflammasome can modulate the intensity of inflammatory responses [78]. Additionally, some natural compounds, such as DIM, can alleviate inflammatory responses by regulating the AhR/NF-κB signaling pathway [79]. Drugs targeting GSDMD and MLKL also hold promise as new therapeutic approaches for inflammation-related diseases [80]. Future research should focus on elucidating the interactions between pyroptosis and other forms of cell death (such as apoptosis, necroptosis, and ferroptosis), as well as developing more precise therapeutic approaches targeting pyroptosis.

2.3 Necrosis

Necrosis is a form of cell death previously considered a non-programmed, accidental event. However, it is now known that necrosis can also occur through regulated pathways, referred to as regulated necrosis, such as necroptosis [81]. Therefore, currently, cell death is broadly classified into accidental necrosis and PCD, with necroptosis being a subtype of PCD rather than a category parallel to accidental necrosis. In this framework, necrosis, in a broad sense, includes two forms. One is accidental necrosis in the traditional sense, which is uncontrollable; the other is regulated necroptosis. Necrosis differs from apoptosis, in that apoptosis is a form of programmed cell death that does not induce an inflammatory response, whereas necrosis is usually associated with inflammation. Accidental necrosis is usually caused by external factors, such as physical injury, chemical substances, or ischemia [82]. These factors lead to the loss of cell membrane integrity, resulting in the uncontrolled release of cellular contents into the extracellular environment. These released cellular contents, including DAMPs, can activate immune cells, leading to inflammatory responses [83]. In contrast, regulated necrotic pathways such as necroptosis represent programmed cell death pathways that can also lead to inflammation in some cases. Unlike accidental necrosis, these regulated pathways are triggered by specific molecular signaling pathways. TNF is one of the most extensively studied triggers of necroptosis [84]. When cells fail to initiate apoptosis, TNF binds to its receptor, activating RIPK1. RIPK1 recruits and activates RIPK3, forming a complex known as the necrosome. RIPK3 phosphorylates MLKL, leading to MLKL oligomeriza-

tion and translocation to the plasma membrane. MLKL oligomerization in the plasma membrane results in membrane rupture and the release of cellular contents, thereby leading to inflammation [85].

Inflammation is a key outcome of both accidental necrosis and regulated necrotic pathways, but the two modes of cell death trigger inflammation through different mechanisms. In accidental necrosis, inflammation is primarily caused by the non-specific release of cellular contents. DAMPs, such as heat shock proteins and HMGB1, can bind to PRRs on immune cells, such as Toll-like receptors (TLRs), thereby activating inflammatory pathways [83]. In regulated necrotic pathways, inflammation is driven by specific molecular mediators released in a controlled manner. For example, MLKL-mediated plasma membrane rupture leads to the release of cytokines such as IL-1β and IL-18, which are potent inflammatory inducers [86]. Furthermore, regulated necrotic pathways can promote inflammation by activating inflammasomes, which are multiprotein complexes in the cytoplasm that activate caspase-1 and lead to the maturation of IL-1β and IL-18 [87]. There are multiple types of programmed cell death, including necroptosis, pyroptosis, and ferroptosis, which lead to inflammation through different molecular mechanisms [88, 89]. Necroptosis is primarily mediated by RIPK1, RIPK3, and MLKL. When caspases are inhibited, RIPK1 and RIPK3 form a necrosome, leading to MLKL phosphorylation and translocation to the plasma membrane, ultimately resulting in membrane rupture and the release of inflammatory mediators. Pyroptosis, on the other hand, is mediated by the gasdermin protein family. Inflammasomes activate caspase-1, which cleaves GSDMD. The GSDMD-N fragment forms pores in the plasma membrane, releasing cellular contents and inflammatory factors. Ferroptosis is a form of cell death driven by iron-dependent lipid peroxidation, leading to the production of ROS and the release of lipid peroxidation products, which can activate immune cells and promote inflammation. Overall, accidental necrosis primarily triggers inflammation through the non-specific release of cellular contents, while regulated necrotic pathways release cellular contents and inflammatory mediators in an orderly manner through the activation of regulatory proteins and the transmission of signaling pathways, thereby initiating a more precise immune response.

Regulated necrotic pathways, as forms of programmed cell death, play a complex and crucial role in the initiation and progression of various diseases. They are characterized by the rupture of the cell membrane and the release of cellular contents, events that trigger inflammatory responses and immune activation. These cell death pathways are associated with various pathological processes, including but not limited to infectious diseases, neurodegenerative diseases, cancer, and cardiovascular diseases [90]. In infectious diseases, regulated necrotic pathways can serve as a defense mechanism by inducing the death of infected cells to limit the spread of pathogens and activate the immune system to clear pathogens [91]. However, regulated necrotic death may also lead to excessive inflammatory responses and tissue damage, exacerbating the severity of the disease. In neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease, regulated necrotic pathways are believed to be involved in the death of neurons, thereby leading to cognitive dysfunction and motor disorders [92, 93]. In cardiovascular diseases, such as myocardial infarction, regulated necrotic pathways may lead to the death of cardiomyocytes and impaired myocardial function [94]. In the tumor microenvironment, regulated necrotic pathways can act as a pro-tumor mechanism, promoting tumor growth and metastasis through inflammation, or as an anti-tumor mechanism. For example, they can be harnessed by integrating natural compounds such as polyphenols, alkaloids, and terpenes to induce tumor cell death, thereby inhibiting tumor progression [95-97]. Intervention strategies targeting regulated necrotic pathways have become a research hotspot. Recent studies have shown that targeting necroptosis can induce anti-tumor immunity, which is crucial for improving cancer treatment [98]. Immunogenic cell death (ICD) can eliminate residual cancer cells by activating the immune system, and necroptosis is a form of ICD. Therefore, therapeutic strategies targeting necroptosis can enhance anti-tumor immune responses and improve therapeutic efficacy. Meanwhile, RIPK3 plays a role in the crosstalk between cell death and inflammation, interacting with RIPK1 to form a necrosome, thereby triggering caspase-independent programmed necrosis [91]. RIPK3 is involved in various infections, aseptic inflammatory diseases, and the pathological processes of tumors, and thus has also become a potential therapeutic target.

A particularly relevant example of the detrimental effects of excessive necroptosis is its contribution to intestinal barrier dysfunction and postoperative infection susceptibility. The intestinal epithelial barrier, maintained by a monolayer of enterocytes and tight junctions, is critical for preventing bacterial and toxin translocation. Perioperative stressors such as surgical trauma, gut ischemia-reperfusion injury, or sepsis can trigger RIPK1/RIPK3/MLKL-mediated necroptosis in enterocytes [99, 100]. Central to this process is the oligomerization of phosphorylated MLKL, which forms pores in the plasma membrane, leading to the lysis of enterocytes. This "lytic" cell death directly disrupts the physical continuity of the epithelial layer, compromising tight junction function and increasing intestinal permeability [101]. The resulting barrier failure allows gut-derived bacteria and PAMPs (e.g., LPS) to enter the systemic circulation, potentially initiating or amplifying systemic inflammatory response syndrome (SIRS) and sepsis. In conditions like necrotizing enterocolitis and inflammatory bowel disease (IBD), upregulated RIPK3/MLKL expression and enterocyte necroptosis are observed. Conversely, inhibiting this pathway (e.g., via RIPK1 inhibitors or compounds like curcumin) alleviates intestinal damage in models. Thus, targeting necroptosis emerges as a promising strategy to preserve intes-

tinal barrier integrity and reduce the risk of postoperative, gut-originated infections [102].

2.4 Ferroptosis

Ferroptosis is a form of cell death driven by iron-dependent lipid peroxidation, distinct from other forms of cell death, as it does not rely on caspase activation and membrane pore formation [103, 104]. The core molecular mechanism of ferroptosis is regulated through two pathways. Among them, the GPX4-dependent pathway plays a central role. GPX4 utilizes glutathione (GSH) as a coenzyme and is responsible for reducing intracellular lipid peroxides to maintain redox balance [105]. When GPX4 activity is directly inhibited (e.g., by the action of RSL3, sorafenib) or when GSH supply is insufficient, lipid peroxides cannot be effectively cleared, leading to their accumulation and driving ferroptosis [106]. GSH synthesis depends on cystine uptake, which is mediated by the cystine/glutamate antiporter system Xc^- (composed of SLC7A11 and SLC3A2) [107]. Compounds such as Erastin inhibit system Xc^-, reduce cystine uptake, thereby inhibit GSH synthesis, and indirectly affect GPX4 function. Another important pathway involves dysregulation of iron metabolism [108]. Intracellular iron exists in two forms: Fe^{2+} and Fe^{3+}. Transferrin binds Fe^{3+}, and this complex enters cells via TFR1. After entering the cell, iron can be stored in ferritin. Intracellular iron ions (especially Fe^{2+}) generate a large amount of ROS through the Fenton reaction, directly driving lipid peroxidation [109]. Notably, autophagic degradation of ferritin (ferritinophagy) releases iron ions, exacerbating ferroptosis [110]. Furthermore, several key metabolic regulatory hubs are crucial for the occurrence of ferroptosis. System Xc^-, serving as a gateway for cystine uptake, directly affects downstream GSH synthesis and GPX4 activity through its functional status [109]. ACSL4 functions at the level of lipid metabolism. It converts arachidonic acid (AA) into AA-CoA, which then participates in the formation of phosphatidylethanolamine-AA, thereby providing substrates for lipoxygenases (ALOXs) to catalyze lipid peroxidation [111]. ALOXs directly catalyze the oxidation of these phospholipids, generating lipid hydroperoxides, ultimately leading to cell membrane damage and ferroptosis. Therefore, system Xc^-, ACSL4, and ALOXs collectively constitute key metabolic nodes that regulate ferroptosis sensitivity.

Ferroptosis, as a form of cell death driven by iron-dependent lipid peroxidation, has complex interactions with inflammatory responses. During ferroptosis, lipid peroxidation generates abundant lipid peroxidation products, such as 4-HNE and MDA [112]. These substances, acting as DAMPs, can activate the TLR4/NF-κB pathway. When TLR4 is activated by DAMPs, it initiates the NF-κB signaling pathway, leading to increased expression and secretion of downstream inflammatory factors, such as IL-6 and TNF-α [113]. These inflammatory factors further exacerbate inflammatory responses and affect the function of the immune system. In addition, mitochondrial damage is also an important factor in ferroptosis-triggered inflammation. During ferroptosis, mitochondrial function is impaired, leading to mtDNA leakage into the cytoplasm. Cytoplasmic mtDNA is recognized by cyclic GMP-AMP synthase (cGAS), activating the cGAS-STING pathway [114]. Upon STING activation, this pathway promotes the release of type I interferons, further enhancing the inflammatory response. In cancer, STING activation can promote anti-tumor immunity by enhancing antigen presentation, recruiting immune cells, and inhibiting metastasis.

A significant disease context linking ferroptosis to inflammation and cellular damage is POCD, which is particularly relevant in elderly patients. Surgical stress and anesthesia can trigger central nervous system inflammation and oxidative stress, especially in the hippocampus, leading to impaired GPX4 function and subsequent neuronal ferroptosis [115]. In POCD animal models, decreased GPX4 expression/activity, along with elevated lipid peroxidation markers (e.g., MDA), iron accumulation, and GSH depletion, is commonly observed [116]. This GPX4 dysfunction-driven ferroptosis not only causes direct neuronal damage but also interacts with neuroinflammation and calcium dyshomeostasis, forming a vicious cycle that accelerates cognitive decline. For instance, GPX4 inhibition can enhance ryanodine receptor-mediated calcium release, further promoting ferroptosis, while damage-associated molecules released during ferroptosis exacerbate neuroinflammation. Conversely, overexpression of GPX4 or its transcriptional regulator MEF2C can suppress hippocampal ferroptosis, alleviate neuroinflammation, and improve cognitive performance in models [117]. Consequently, targeting the GPX4-ferroptosis axis is a promising strategy for POCD intervention. Approaches such as using electroacupuncture, which may modulate iron metabolism proteins like TFR1, DMT1, and FPN, as well as supplying GPX4 substrates (e.g., N-acetylcysteine), using iron chelators or lipophilic radical-trapping antioxidants, and applying neuroprotective near-infrared light, have shown anti-ferroptotic and cognitive protective effects in preclinical studies [118]. Furthermore, regulating mitochondrial calcium homeostasis (e.g., via MICU1 overexpression) or inhibiting ferritinophagy may also influence neuronal ferroptosis sensitivity [119].

Given the critical role of ferroptosis in the pathological processes of various diseases, researchers are actively developing targeted intervention strategies, which have shown potential in preclinical studies. In the field of neurodegenerative diseases, such as Parkinson's disease, ferroptosis has been confirmed to be involved in the damage process of dopaminergic neurons [120]. The application of GPX4 agonists such as Liproxstatin-1 can effectively reduce the accumulation of lipid peroxides within neurons, thereby inhibiting ferroptosis and providing a potential pathway to delay the progression of such diseases. For myocardial ischemia-reperfusion injury, ferroptosis similarly exacerbates the death of cardiomyocytes [121]. In this context,

the use of iron chelators (such as deferoxamine) to reduce intracellular unstable iron ion levels and inhibit free radical generation has become an effective strategy for mitigating myocardial ferroptosis and tissue damage. Furthermore, regulating the expression or activity of key ferroptosis pathway proteins such as ACSL4 and GPX4 has also been proven to protect cardiac function [122, 123]. In tumor therapy, inducing cancer cells to undergo ferroptosis has shown unique value. Studies have found that promoting ferroptosis in tumor cells can significantly enhance the efficacy of immunotherapy, for example, by increasing the sensitivity of tumors to immune checkpoint inhibitors such as PD-1 antibodies [124]. Therefore, the combined application of ferroptosis inducers and immune checkpoint inhibitors, aiming to enhance the body's anti-tumor immune response through a dual mechanism, is considered a synergistic anti-tumor strategy with broad prospects.

2.5 Cuproptosis

Cuproptosis is a programmed cell death first reported by Tsvetkov's team in *Science* in 2022. It is triggered by excessive copper ions (Cu^{2+}) and specifically targets the mitochondrial respiratory chain. Its key molecular mechanism involves the binding of copper to lipoylated tricarboxylic acid cycle enzymes, leading to protein aggregation and cell death [125]. Cuproptosis causes proteotoxic stress and reduces iron-sulfur cluster proteins through copper-dependent abnormal oligomerization reactions, a process that distinguishes it from other forms of cell death. When copper ion concentration exceeds the physiological threshold, excessive copper ions specifically bind to FDX1 and its downstream proteins in the mitochondrial respiratory chain. This binding leads to the loss of function of the key metabolic enzyme DLAT and promotes the oligomerization of these target proteins [126]. Oligomerized proteins further form insoluble aggregates intracellularly (especially in mitochondria), triggering severe proteotoxic stress. Ultimately, this series of events disrupts normal mitochondrial function, particularly interfering with the tricarboxylic acid cycle and respiratory chain electron transport processes, leading to cellular energy metabolism collapse and irreversible death.

Cuproptosis is closely related to mitochondrial metabolism, and copper ion overload can lead to the release of mitochondrial components, which include DAMPs and cytokines [127]. DAMPs and cytokines can activate immune cells, leading to sterile inflammation, which in turn causes tissue damage and remodeling. Furthermore, copper ions can participate in redox reactions, leading to the production of ROS, thereby triggering oxidative stress [128]. Oxidative stress is a state of intracellular imbalance between oxidants and antioxidants, where excessive oxidants can cause damage to cells and activate inflammatory responses. This is similar to the mechanism of ferroptosis-induced inflammation. Meanwhile, both ferroptosis and cuproptosis play significant roles in tumor cell proliferation, metastasis, and drug resistance [129, 130]. Ferroptosis is associated with chemoresistance, and inducing ferroptosis may be a method to address chemoresistance. Furthermore, cuproptosis exhibits a chemosensitizing effect in cancer and can overcome chemoresistance. For example, copper ionophores can be used in combination with chemotherapeutic drugs to increase the intracellular copper ion concentration in cancer cells, thereby inducing cuproptosis and enhancing chemosensitivity [131]. Meanwhile, studies have shown that some cancer cells that develop resistance to traditional chemotherapy drugs are still sensitive to cuproptosis [132]. This makes cuproptosis-based cancer therapy a new research focus. Furthermore, cuproptosis can be used in combination with other cancer treatment modalities to achieve better therapeutic effects. For example, cuproptosis can be combined with immunotherapy to enhance tumor immunogenicity and improve the efficacy of immunotherapy [133]. However, copper ions can promote PD-L1 expression and tumor immune evasion, giving cuproptosis a dual nature in immunotherapy. In the future, the use of copper chelators to target and regulate intracellular copper ion homeostasis in tumor cells is expected to provide new ideas and methods for tumor immunotherapy.

2.6 Autophagy

Autophagy is a lysosome-mediated degradation process of cytoplasmic components, playing a dual role in cell fate. Under basal conditions, autophagy acts as a cell protective mechanism by clearing damaged organelles, misfolded proteins, and invading pathogens to maintain cellular homeostasis [134]. However, under specific conditions, such as sustained stress stimuli or regulatory imbalance, autophagy may be excessively activated, leading to autophagy-dependent cell death, also known as autophagic cell death [135]. The molecular pathway of autophagic cell death differs from traditional apoptosis and necrosis, and its morphological features include cytoplasmic vacuolization and nuclear condensation [136]. Autophagy's dual functions depend on the precise regulation of its core molecules, such as Beclin1 and ATG family proteins [31]. The transition of autophagy from a protective mechanism to a death-promoting function is influenced by various factors, including excessive stress and the activation of specific molecular switches, while the dependence on ATG5/ATG7 determines the occurrence of autophagic cell death.

The molecular regulatory mechanism of autophagy is complex and intricate, involving multiple key proteins and protein complexes, among which the ULK1 complex and PI3K complex play a central role in the autophagy initiation phase [137]. The ULK1 complex is composed of ULK1, ATG13, FIP200, and ATG101, and the activation of this complex is regulated by AMPK and mTOR [138]. Under nutrient-rich conditions, mTORC1 inhibits the ULK1 complex, thereby inhibiting autophagy [139]. Conversely, under conditions of nutrient deprivation or cellular stress, AMPK is activated and phosphorylates the ULK1 complex, thereby initiating autophagy

[140]. The PI3K complex, comprising Beclin1, Vps34, ATG14, and Vps15, is responsible for generating PI3P, which is crucial for phagophore formation [141]. During autophagosome formation, two ubiquitin-like conjugation systems play crucial roles [142]. Firstly, the formation of the ATG12-ATG5-ATG16L1 complex promotes the lipidation of LC3. LC3 is microtubule-associated protein 1 light chain 3, and its lipidated form, LC3-II, binds to the autophagosomal membrane, facilitating autophagosome formation. Although LC3 lipidation is widely used as an indicator of autophagy, it is absent in ATG5/ATG7-independent alternative autophagy [143]. This alternative pathway forms autophagosomes through the fusion of Rab9-mediated phagophores with trans-Golgi network/late endosome-derived vesicles [144]. This reveals that mammalian autophagy exists in two main forms: the classical ATG5/ATG7-dependent pathway and the alternative Rab9-dependent pathway. The fusion of autophagosomes with lysosomes is the final stage of the autophagic process. This fusion event is mediated by SNARE proteins, forming autolysosomes [145]. Inside the autolysosomes, lysosomal enzymes degrade the sequestered cellular material and release degradation products such as amino acids, fatty acids, and nucleotides back into the cytoplasm for cellular reuse.

There are complex interactions between autophagy and other forms of cell death such as apoptosis and necrosis. Autophagy can affect the occurrence of apoptosis through multiple pathways. For example, autophagy can clear mitochondria, thereby inhibiting mitochondria-mediated apoptosis [31]. In addition, autophagy can degrade apoptosis-related proteins such as caspase, thereby inhibiting the occurrence of apoptosis. However, in gastric cancer, autophagy can also promote apoptosis [146]. Cleavage of Beclin1 is considered an important molecular switch for the autophagy-to-death transition. Caspase-8 can cleave Beclin1, thereby inhibiting the protective function of autophagy and potentially promoting apoptosis [147]. Furthermore, cleavage of Beclin1 may release pro-apoptotic factors, such as Bax, further promoting cell death. The relationship between autophagy and necrosis is also highly complex. Necrosis is a form of non-programmed cell death, usually resulting from severe physical or chemical damage to cells. Autophagy can inhibit the occurrence of necrosis by clearing damaged organelles and protein aggregates [148]. However, autophagy can also lead to cellular energy depletion by degrading energy substances such as ATP, ultimately resulting in the occurrence of necrosis [149].

Autophagy plays a complex bidirectional regulatory role in the inflammatory response. On the one hand, autophagy can inhibit inflammation by clearing intracellular damaged substances and pathogens. It can also degrade damaged mitochondria and reduce the production of ROS, thereby alleviating oxidative stress and inflammatory responses [150]. In addition, autophagy can clear misfolded proteins and aggregates, preventing them from triggering inflammatory responses. On the other hand, autophagy can also enhance inflammatory signals by selectively degrading certain anti-inflammatory molecules [151]. It is also involved in the activation of inflammasomes, promoting the release of inflammatory factors. Due to the complexity of autophagy in inflammatory responses, it plays a dichotomous role in various diseases, having both protective and pathogenic effects. In Alzheimer's disease, autophagy can promote the clearance of β-amyloid protein, but overactivated autophagy may also lead to neuronal damage [152]. In IBD, autophagy plays an important role in maintaining intestinal homeostasis and regulating gut microbiota. Autophagy of intestinal macrophages can clear pathogens and damaged cells in the intestine, reducing inflammatory responses [153]. Studies have found that, in Crohn's disease, mutations in the ATG16L1 gene lead to autophagy dysfunction, increasing the risk of intestinal inflammation [154]. However, autophagy may promote macrophage M1 polarization, and M1 macrophages primarily produce pro-inflammatory factors, exacerbating the inflammatory response [155]. Given the complex role autophagy plays in inflammation and disease, therapeutic strategies targeting autophagy require careful consideration. Currently, there are many drugs and natural compounds that can modulate autophagy activity. For example, Rapamycin is an mTOR inhibitor that can enhance autophagy [156]. Flavonoids, such as quercetin, can promote autophagy by activating the AMPK pathway [157]. Furthermore, traditional Chinese medicine has shown potential in modulating autophagy and inflammation. Traditional Chinese medicine, especially Fangji Huangqi Decoction, has been used to treat rheumatoid arthritis (RA) [158]. Future research needs to further elucidate the specific mechanisms of autophagy in different diseases, providing a theoretical basis for developing more effective therapeutic strategies.

2.7 Other types of cell death

NETosis and PANoptosis are both important cell death modalities in inflammatory responses, but they exhibit significant differences in mechanisms and functions. NETosis is primarily executed by neutrophils to capture and kill pathogens, but excessive activation can lead to inflammation and tissue damage [159]. PANoptosis, however, is a more complex cell death modality, integrating multiple pathways of apoptosis, pyroptosis, and necroptosis, playing important roles in both immune defense and inflammatory responses. NETosis is a neutrophil-specific programmed cell death. This process involves the release of web-like structures by neutrophils. These structures, known as neutrophil extracellular traps (NETs), contain DNA, histones, and granular proteins [160]. The primary function of NETs is to trap and kill pathogens, thereby limiting the spread of infection. However, excessive NETosis can lead to the accumulation of NETs in tissues, inducing inflammatory responses, and it is associated with various diseases, including autoimmune diseases, thrombosis, and organ damage [161]. On the other hand, PANoptosis is a newly discovered inflammatory

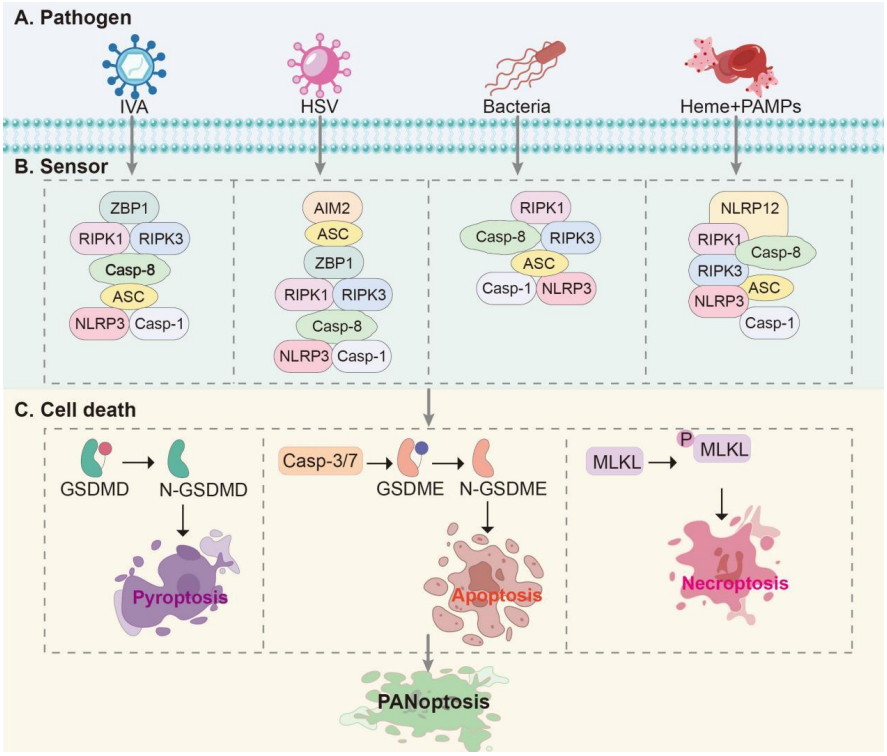

Figure 4. Pathogen-specific activation of integrated cell death pathways via distinct sensor molecules. Schematic model illustrating how diverse pathogens engage specific sensor proteins to trigger an integrated cell death response. (A) Pathogens. Various pathogenic stimuli, including IVA, HSV, bacteria, and heme together with PAMPs, can initiate the signaling cascade. (B) Sensors. Each pathogen or stressor is detected by specific intracellular or extracellular sensor molecules, such as ZBP1 for IAV, AIM2 for viral/bacterial DNA, and RIPK1 for inflammatory signaling. These sensors act as the core molecular platform for signal integration. (C) Cell Death Execution. Activation of the sensor complexes leads to the recruitment and activation of key effector molecules, culminating in the induction of pyroptosis (executed by cleaved GSDMD and its N-terminal fragment, N-GSDMD), apoptosis (mediated by activated Casp-3/7), and/or necroptosis (executed by p-MLKL). This model demonstrates the concept of PANoptosis, where a single sensor complex can coordinately regulate multiple cell death modalities to mount a robust host defense. IVA, Influenza A virus; HSV, Herpes Simplex virus; PAMPs, pathogen-associated molecular patterns; ZBP1, Z-DNA-binding protein 1; AIM2, absent in melanoma 2; RIPK1, receptor-interacting protein kinase 1; RIPK3, receptor-interacting protein kinase 3; NLRP12, NLR family pyrin domain containing 12; NLRP3, NLR family pyrin domain containing 3; ASC, apoptosis-associated speck-like protein containing a CARD; Casp-8, caspase-8; Casp-1, caspase-1; MLKL, mixed lineage kinase domain-like pseudokinase; Casp-3/7, caspase-3/7; GSDMD, gasdermin D; N-GSDMD, N-terminal fragment of gasdermin D; GSDME, gasdermin E; N-GSDME, N-terminal fragment of gasdermin E; p-MLKL, phosphorylated mixed lineage kinase domain-like pseudokinase.

programmed cell death pathway that integrates the features of apoptosis, pyroptosis, and necroptosis. The activation of PANoptosis is typically mediated by the PANoptosome complex, which contains various proteins capable of responding to pathogen or damage-associated signals [162]. While pathogens are being cleared, PANoptosis also releases a large number of inflammatory mediators, thereby playing a role in the pathophysiological processes of various diseases, such as infection, autoimmune diseases, and cancer.

The initiation of NETosis is usually triggered by PAMPs or DAMPs, which bind to receptors on the surface of neutrophils, activating intracellular signaling pathways [160]. Subsequently, ROS are produced in large quantities. The production of ROS is a critical step in the process of NETosis. ROS promote chromatin decondensation and the rupture of the cell membrane by oxidizing cellular components [163]. PAD4 mediates histone citrullination, leading to chromatin decondensation and creating conditions for the formation of NETs [164]. Ultimately, the neutrophil cell membrane ruptures, releasing NETs containing DNA, histones, and granular proteins. The DNA and granular proteins in NETs possess antimicrobial activity, directly killing pathogens [165]. Therefore, the primary function of NETs is to capture and kill pathogens such as bacteria and fungi, thereby limiting the spread of infection. However, NETs can interact with monocytes/macrophages, stimulating them to release inflammatory factors, such as IL-1β and TNF-α [166]. Furthermore, NETs can activate the complement system, further amplifying the inflammatory response [167]. Moreover, proteases in NETs (such as neutrophil elastase) can directly destroy tissue structures. Excessive NETosis can lead to the deposition of NETs in tissues, causing tissue damage [168]. This is also why NETosis is considered a culprit in various inflammatory diseases. The role of NETosis in autoimmune diseases will be elaborated in detail later in this text.

PANoptosis can be triggered by various signals, including PAMPs, DAMPs, and cytokines. These signals are recognized by cells through different PRRs, initiating downstream signaling pathways. Upon signal activation, PANoptosome complexes form within cells [169]. Different PANoptosome complexes contain different sensors and adaptor proteins, such as ZBP1, AIM2, and RIPK1 [21]. Upon PANoptosome activation, the execution mechanisms of apoptosis, pyroptosis, and necroptosis are simultaneously initiated. This includes caspase activation, GSDMD-mediated cell membrane perforation, and MLKL-mediated cell membrane disruption [170]. All processes are shown in **Figure 4**. PANoptosis can regulate inflammatory responses through multiple pathways. On the one hand, PANoptosis can induce the death of infected cells, thereby clearing pathogens and limiting infection spread.

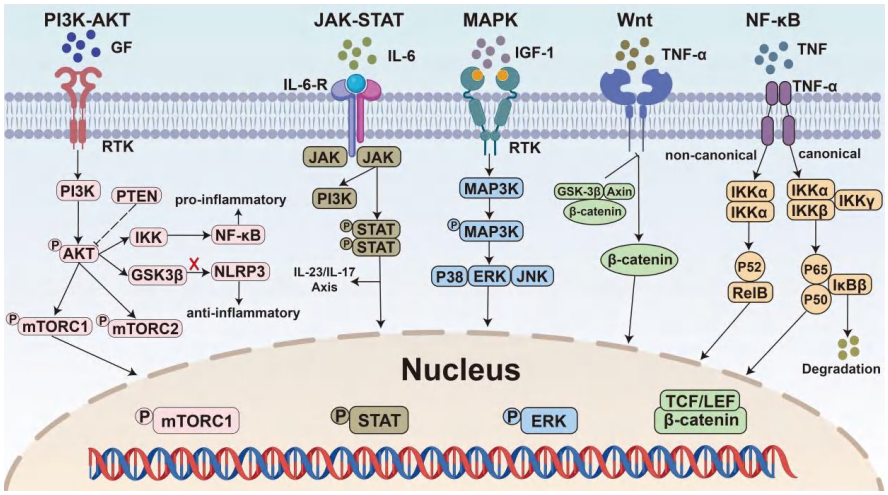

Figure 5. An integrated network of core signaling pathways governing inflammation and cell fate. Schematic model depicting the interplay between five pivotal signal transduction pathways: PI3K-AKT, JAK-STAT, MAPK, Wnt, and NF-κB. The diagram is spatially organized with the plasma membrane (top) and nucleus (bottom) to illustrate the signal flow from extracellular ligand-receptor binding to nuclear gene regulation. Key ligands, including GFs, cytokines (e.g., IL-6, TNF-α), and others, bind to their respective receptors (e.g., RTKs, IL-6 receptor), initiating intracellular cascades. The model highlights critical pathway components, their activation (e.g., phosphorylation of AKT, STAT, ERK, IκB), and nuclear translocation of transcription factors (e.g., NF-κB, STAT, β-catenin). Importantly, it emphasizes extensive cross-talk between pathways, such as the regulatory node of GSK3β, the IL-23/IL-17 axis, and feedback mechanisms (e.g., PTEN inhibition of PI3K). This integrated view provides a framework for understanding how cells coordinate diverse signals to regulate survival, proliferation, inflammatory responses, and gene expression programs. PI3K, phosphoinositide 3-kinase; AKT, protein kinase B; JAK, Janus kinase; STAT, signal transducer and activator of transcription; MAPK, mitogen-activated protein kinase; Wnt, Wingless/Integrated; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; GF, growth factor; IL-6, interleukin-6; IGF-1, insulin-like growth factor 1; TNF-α, tumor necrosis factor-alpha; TNF, tumor necrosis factor; IL-6-R, interleukin-6 receptor; RTK, receptor tyrosine kinase; PTEN, phosphatase and tensin homolog; PI3K, phosphoinositide 3-kinase; MAP3K, mitogen-activated protein kinase kinase kinase; GSK-3β, glycogen synthase kinase-3β; IKK, inhibitor of nuclear factor kappa-B kinase; IKKα, inhibitor of nuclear factor kappa-B kinase subunit alpha; IKKβ, inhibitor of nuclear factor kappa-B kinase subunit beta; IKKγ, inhibitor of nuclear factor kappa-B kinase subunit gamma; mTORC1, mechanistic target of rapamycin complex 1; mTORC2, mechanistic target of rapamycin complex 2; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; P38, p38 mitogen-activated protein kinase; RelB, v-rel reticuloendotheliosis viral oncogene homolog B; P50, NF-κB p50 subunit; P52, NF-κB p52 subunit; P65, NF-κB p65 subunit; IκB, inhibitor of NF-κB; NLRP3, NLR family pyrin domain containing 3; IL-23, interleukin-23; IL-17, interleukin-17; TCF, T-cell factor; LEF, lymphoid enhancer-binding factor.

On the other hand, PANoptosis also releases a large number of inflammatory mediators, exacerbating the inflammatory response [171]. In pulmonary arterial hypertension, pulmonary vascular remodeling is the main pathological change, and PANoptosis is closely related to the abnormal death of pulmonary vascular cells and perivascular inflammation [172]. Activation of PANoptosis leads to immune cell adhesion and inflammatory mediator release, further exacerbating vascular remodeling.

3 REGULATORY NETWORK OF INFLAMMARY RESPONSE

Inflammation is a complex biological response made by the body to injury or infection, aimed at eliminating harmful stimuli and initiating tissue repair processes. This process involves various cell types, molecules, and signaling pathways, requiring precise regulation to prevent excessive inflammatory responses, which can lead to further tissue damage and chronic diseases [173]. The triggering of inflammation relies on the molecular recognition of danger signals and the complex mechanisms of action of inflammatory mediators. The body recognizes PAMPs and DAMPs through PRRs. PAMPs are conserved structures shared by microorganisms, such as LPS, peptidoglycan, and flagellin, indicating the presence of infection [174]. DAMPs are endogenous molecules released when cells are damaged or die, including HMGB1, heat shock proteins, and ATP, suggesting tissue damage or cellular stress [175, 176]. PRRs include TLRs, NOD-like receptors (NLRs), C-type lectin receptors (CLRs), and RIG-I-like receptors (RLRs) [177-179]. For example, TLR4 recognizes LPS, activating MyD88-dependent and -independent signaling pathways, leading to the activation of NF-κB and IRF3, ultimately promoting the production of pro-inflammatory cytokines and type I interferons. NLRs are intracellular receptors, recognizing PAMPs and DAMPs within the cytoplasm. The NLRP3 inflammasome is the most extensively studied NLR, which can be activated by various stimuli, including ATP, urate crystals, and amyloid-beta, leading to the activation of caspase-1 and the release of IL-1β and IL-18. CLRs primarily recognize carbohydrate structures on the surface of pathogens and are involved in antifungal immunity. RLRs recognize viral RNA, activate MAVS-mediated signaling pathways, and induce the production of type I interferons, thereby resisting viral infection.

After PRR activation, downstream signaling pathways are activated, leading to the production and release of inflammatory mediators. Inflammatory signaling pathways can be divided into classical and non-classical pathways, which interact with each other to jointly regulate various aspects of the inflammatory response, as shown in **Figure 5**. Classical inflammatory

signaling pathways play a central role in regulating inflammatory responses, mainly including the NF-κB pathway, JAK-STAT pathway, and MAPK pathway. The NF-κB pathway is a key regulatory factor in inflammatory responses [180]. Various stimuli, such as pathogens, pro-inflammatory cytokines (e.g., TNF-α, IL-1β), and oxidative stress, can activate the NF-κB pathway. After ligands bind to TLR receptors on the cell surface, intracellular signal transduction is activated, leading to the activation of the IκB kinase complex. The activated IκB kinase complex phosphorylates IκB proteins, leading to IκB protein degradation and the release of NF-κB dimers (typically p65/p50). The released NF-κB dimers then translocate into the cell nucleus and bind to the promoter regions of target genes. This binding regulates the transcription of downstream genes, including pro-inflammatory cytokines (such as TNF-α, IL-1β, IL-6), chemokines, and adhesion molecules. Activation of the NF-κB pathway plays a crucial role in the initiation and amplification of inflammatory responses. Recent studies have shown that overactivation of the NF-κB pathway is closely associated with the development and progression of various diseases, including atherosclerosis, IBD, and cancer [181, 182]. Negative regulation of NLRs can alleviate inflammation through the NF-κB signaling pathway in IBD. Therefore, the NF-κB pathway is an important target for treating inflammation-related diseases. The JAK-STAT pathway is the main route for cytokine signaling. After cytokines (e.g., interferons, interleukins) bind to cell surface receptors, receptor-associated JAK kinases are activated [183]. Activated JAK kinases phosphorylate STAT proteins, leading to STAT protein dimerization and translocation into the nucleus. In the nucleus, STAT dimers bind to target gene promoters, regulating gene transcription. The JAK-STAT pathway is involved in regulating various immune and inflammatory responses, including cell proliferation, differentiation, and apoptosis. Studies have shown that the JAK-STAT pathway plays an important role in the pathogenesis of chronic inflammatory diseases (such as RA and IBD) [184, 185]. Therefore, inhibiting the JAK-STAT pathway is one of the effective strategies for treating these diseases. The MAPK pathway is a conserved signal transduction pathway involved in regulating various cellular physiological processes, including cell growth, differentiation, stress response, and inflammation [112]. The MAPK pathway includes three major sub-pathways: ERK, JNK, and p38 MAPK. These sub-pathways are activated by various intracellular and extracellular stimuli, such as growth factors, cytokines, oxidative stress, and ultraviolet radiation. The activated MAPK pathway regulates gene transcription and protein synthesis by phosphorylating downstream target proteins, thereby affecting inflammatory responses.

In addition to classical pathways, non-classical inflammatory signaling pathways play important roles in inflammation regulation, primarily including the NLRP3 inflammasome pathway and the Wnt/β-catenin pathway. The NLRP3 inflammasome is an intracellular multiprotein complex that plays a crucial role in innate immune responses [186]. It is composed of NLRP3 protein, adaptor protein ASC, and effector molecule caspase-1 [187]. The activated NLRP3 inflammasome recruits ASC protein and pro-caspase-1 to form a complex, activating caspase-1. Activated caspase-1 cleaves pro-IL-1β and pro-IL-18, generating mature IL-1β and IL-18, which are then released into the extracellular space. IL-1β and IL-18 are potent pro-inflammatory cytokines that can further activate immune cells and promote inflammatory responses. Overactivation of the NLRP3 inflammasome is associated with various inflammatory diseases, including autoimmune diseases and metabolic diseases, which will be discussed in detail in Section 5 [188]. The Wnt/β-catenin pathway was initially thought to play a role in embryonic development and cell differentiation, but recent studies have found that it also plays an important role in inflammatory responses [189]. In the absence of Wnt ligands, β-catenin protein in the cytoplasm is phosphorylated by a complex consisting of antigen-presenting cell (APC), Axin, and GSK-3β, and subsequently ubiquitinated for degradation. When Wnt ligands bind to Frizzled receptors on the cell surface, this binding activates Dishevelled protein, inhibiting the activity of the β-catenin degradation complex, leading to the accumulation of β-catenin in the cytoplasm. The accumulated β-catenin enters the cell nucleus, binds to transcription factors T-cell factor/lymphoid enhancer-binding factor, activating the transcription of downstream target genes, including genes involved in cell proliferation, differentiation, and inflammation. The Wnt/β-catenin pathway is involved in regulating cytokine production, inflammatory cell infiltration, and tissue remodeling, among other processes. Similar to other inflammatory signaling pathways, the Wnt/β-catenin pathway has been increasingly recognized by researchers for its role in the pathogenesis of chronic inflammatory diseases such as osteoarthritis [190]. Drug development targeting these signaling pathways provides new strategies for treating various inflammation-related diseases.

Inflammatory mediators are key molecules in inflammatory responses, including cytokines, chemokines, lipid mediators, and ROS. They participate in the initiation, amplification, and resolution of inflammatory responses through different mechanisms. Cytokines are small protein molecules produced by immune and non-immune cells, playing various roles in inflammatory responses [191]. Pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 can activate endothelial cells, promote leukocyte recruitment, enhance vascular permeability, and induce the production of other inflammatory mediators. Anti-inflammatory cytokines such as IL-10 and TGF-β inhibit inflammatory responses through mechanisms such as inhibiting the production of pro-inflammatory cytokines and promoting the differentiation of regulatory T cells (Treg), thereby helping inflammation subside and promoting tissue repair [192]. Chemokines are a class of small molecule cytokines whose main function is to guide immune cells to migrate to inflammatory sites. Chemokines, by binding to G protein-coupled receptors on the cell surface, activate intracellular signaling pathways, regulating cell migration, adhesion, and activation [179].

For example, MCP-1/CCL2 can recruit monocytes and macrophages to inflammatory sites, participating in the clearance and repair of inflammatory responses. Lipid mediators are a class of inflammatory mediators derived from the metabolism of unsaturated fatty acids such as AA. AA is produced when phospholipase A2 acts on cell membrane phospholipids. These mediators include prostaglandins, leukotrienes, and lipoxins [193]. Prostaglandins, by activating different prostaglandin receptor subtypes, are involved in regulating inflammatory responses such as vasodilation, vascular permeability, pain, and fever. Leukotrienes are mainly produced by leukocytes and can promote increased vascular permeability, bronchoconstriction, and leukocyte recruitment. Lipoxins, on the other hand, are a class of lipid mediators with anti-inflammatory and pro-resolving effects, capable of inhibiting neutrophil recruitment, promoting macrophage phagocytosis, and accelerating the resolution of inflammation. ROS are a class of oxidatively active molecules produced during inflammatory responses, including superoxide anion, hydrogen peroxide, and hydroxyl radicals, among others [194]. At low concentrations, ROS can act as signaling molecules, participating in the regulation of cell proliferation, differentiation, and apoptosis. However, at high concentrations, ROS can damage intracellular DNA, proteins, and lipids, leading to cell death and tissue damage, and exacerbating inflammatory responses.

The interactions among inflammatory mediators are highly complex, forming an intricate regulatory network. The balance between pro-inflammatory mediators and anti-inflammatory mediators determines the intensity and duration of the inflammatory response. At various stages of the inflammatory response, different types of inflammatory mediators play distinct roles. The initiation and amplification phases of the inflammatory response are primarily dominated by pro-inflammatory mediators, such as TNF-α, IL-1β, and IL-6 [195]. In contrast, inflammation resolution is an active, highly regulated process aimed at eliminating the inflammatory response and restoring tissue homeostasis [196]. As inflammation progresses, pro-inflammatory signaling pathways are gradually suppressed. The activity of the NF-κB pathway is subject to negative feedback regulation, and the production of pro-inflammatory cytokines decreases. The production of anti-inflammatory mediators such as IL-10 and TGF-β increases, further suppressing the pro-inflammatory response. Among these, the production of specialized pro-resolving mediators (SPMs) plays an important role. SPMs are a class of lipid mediators derived from omega-3 fatty acids, including lipoxins, resolvins, protectins, and maresins [197]. SPMs promote neutrophil apoptosis and macrophage phagocytosis, reduce neutrophil recruitment, and inhibit the production of pro-inflammatory cytokines. During inflammation resolution, macrophages polarize from M1 to M2 [198]. M2 macrophages produce anti-inflammatory cytokines such as IL-10 and TGF-β, promoting tissue repair and angiogenesis. Impaired inflammation resolution is closely related to the pathogenesis and progression of various diseases. In atherosclerosis, failed inflammation resolution leads to chronic vascular inflammation, promoting plaque formation and progression [199]. In obesity, persistent adipose tissue inflammation leads to insulin resistance and type 2 diabetes [47]. In summary, the regulatory network of the inflammatory response is a complex and highly coordinated system, involving the synergistic action of multiple signaling pathways and cell types, aiming to achieve effective defense against injury and infection while preventing damage to the body from excessive inflammation.

4 RECIPROCAL REGULATION OF CELL DEATH AND INFLAMMATION

The molecular decoding of cell death-driven inflammatory responses reveals that various cell death modalities release DAMPs. These DAMPs initiate inflammatory responses by binding to PRRs in a mechanism as precise as a "key-lock" match, as shown in **Table 2**. Necroinflammation is the process by which cell death, under physiological or pathological conditions, releases highly immunogenic intracellular molecules and organelles into the interstitial space, thereby triggering a strong immune response [211]. After cell death, the DAMPs released due to the disruption of cell membrane integrity are diverse, including HMGB1, ATP, uric acid, IL-1α, S100 proteins, and mitochondria-derived DAMPs (e.g., mitochondrial DNA, N-formyl peptides) [212]. These molecules have specific physiological functions intracellularly, but upon release to the extracellular space, they act as "danger signals" activating the immune system. Notably, different cell death modalities (e.g., apoptosis, necrosis, pyroptosis, necroptosis) release varying types and quantities of DAMPs, which directly affects the intensity of the inflammatory response. For example, apoptosis typically releases fewer DAMPs, resulting in weak inflammation; whereas necrosis and pyroptosis release large amounts of DAMPs, triggering strong inflammation [17]. Immune cells sense these DAMPs via PRRs. The PRR family includes TLRs, NLRs, RLRs, and CLRs, among others. The binding of DAMPs to specific PRRs is highly selective, similar to a "key-lock" mechanism. For example, HMGB1 and S100 proteins can bind to TLR4, and the HMGB1-TLR4 interaction activates the NF-κB pathway [213]. ATP and uric acid mainly activate the intracellular receptor NLRP3, which in turn triggers inflammasome assembly, caspase-1 activation, and the maturation and release of IL-1β/IL-18 [214]. Mitochondrial DNA, on the other hand, can be recognized by TLR9. This specific recognition ensures the initiation of corresponding immune responses against different damage signals, thereby activating signaling pathways such as NF-κB, MAPK, and IRF, ultimately leading to the production of inflammatory mediators like cytokines and chemokines [215]. This inflammatory microenvironment initiated by the DAMPs-PRRs axis lays the foundation for the subsequent regulation of cell death patterns.

Building upon the aforementioned inflammation initiation mechanisms, key cytokines such as TNF-α and IFN-γ form a

Table 2. Precision recognition of host-derived DAMPs by PRRs and disease cascades

Released DAMPs	Recognition receptor (s)	Downstream pathway	Activated inflammatory mediators	Cell death feedback mechanism	Disease dysregulation	References
HMGB1	TLR4, RAGE	MyD88/ TRIF→NF-κB	TNF-α, IL-6	Necroptosis (RIPK3 activation)	Spinal cord injury	[200]
Mitochondrial DNA (mtDNA)	TLR9, cGAS	STING→IRF3/ NF-κB	IFN-α/β, CXCL10	Mitochondrial apoptosis (BAX/BAK activation)	Lupus nephritis (SLE)	[201]
ATP	P2X7R	NLRP3 inflammasome activation	IL-1β, IL-18	Triggers pyroptosis (GSDMD pore formation)	Gout	[69]
Monosodium urate crystals	NLRP3 (Direct activator)	NLRP3 inflammasome activation	IL-1β, Neutrophil infiltration	Crystal phagocytosis→Lysosomal rupture→Necrosis	Gouty joint erosion	[202]
S100A8/A9	TLR4, RAGE	NF-κB/p38 MAPK	CXCL1, GSDMD pore formation	GSDMD pore formation	RA bone destruction	[203]
Oxidized phospholipids	CD36/TLR4	NLRP3 activation	TNF-α, IL-6	Lipid peroxidation, Ferroptosis	NASH liver fibrosis	[204]
Heat shock protein 70 (HSP70)	TLR2/4, LOX-1	MyD88→NF-κB	CD80/86	Inhibits apoptosis (HSP70-Bax binding)	Tumor immune evasion	[205]
Heparan sulfate fragments	TLR4, CXCR3	TRIF→IRF3	NETs formation→TLR9-IFN-I loop activation	NETs formation	Sepsis microthrombi	[206]
Citrullinated histones	TLR2/4, FcγR	FcγR→Complement activation	Anti-dsDNA	Induces NETosis	Lupus nephritis (SLE)	[207]
Fibronectin fragments	α5β1 integrin	FAK-Src→NF-κB	α-SMA, TGF-β→Induces EMT	Induces EMT	Liver fibrosis	[208]
Mitochondrial cardiolipin	NLRP3	NLRP3 inflammasome activation	IL-1β	Promotes mPTP opening→Initiates apoptosis	Sepsis ARDS	[209]
Endogenous retroviral elements	TLR7, MDA5	MAVS→IRF7	IFN-α	Activates ZBP1-PANoptosis	SLE	[210]

Note: DAMPs, damage-associated molecular patterns; PRRs, pattern recognition receptors; HMGB1, high mobility group box 1; TLR4, Toll-like receptor 4; RAGE, receptor for advanced glycation end products; MyD88, myeloid differentiation primary response 88; TRIF, TIR-domain-containing adapter-inducing interferon-β; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; TNF-α, tumor necrosis factor-alpha; IL-6, interleukin-6; RIPK3, receptor-interacting protein kinase 3; mtDNA, mitochondrial DNA; TLR9, Toll-like receptor 9; cGAS, cyclic GMP-AMP synthase; STING, stimulator of interferon genes; IRF3, interferon regulatory factor 3; IFN-α/β, interferon-alpha/beta; CXCL10, C-X-C motif chemokine ligand 10; BAX, BCL2-associated X protein; BAK, BCL2-antagonist/killer 1; SLE, systemic lupus erythematosus; ATP, adenosine triphosphate; P2X7R, P2X purinoceptor 7; NLRP3, NLR family pyrin domain containing 3; IL-1β, interleukin-1β; IL-18, interleukin-18; GSDMD, gasdermin D; S100A8/A9, S100 calcium-binding protein A8/A9; p38 MAPK, p38 mitogen-activated protein kinase; CXCL1, C-X-C motif chemokine ligand 1; RA, rheumatoid arthritis; CD36, cluster of differentiation 36; NASH, non-alcoholic steatohepatitis; HSP70, heat shock protein 70; TLR2, Toll-like receptor 2; LOX-1, lectin-type oxidized LDL receptor 1; CD80/86, cluster of differentiation 80/86; CXCR3, C-X-C chemokine receptor type 3; NETs, neutrophil extracellular traps; IFN-I, type I interferon; FcγR, Fc gamma receptor; anti-dsDNA, anti-double-stranded DNA antibody; α5β1 Integrin, alpha5 beta1 integrin; FAK, focal adhesion kinase; Src, proto-oncogene tyrosine-protein kinase Src; α-SMA, alpha-smooth muscle actin; TGF-β, transforming growth factor-beta; EMT, epithelial-mesenchymal transition; mPTP, mitochondrial permeability transition pore; ARDS, acute respiratory distress syndrome; TLR7, Toll-like receptor 7; MDA5, melanoma differentiation-associated protein 5; MAVS, mitochondrial antiviral signaling protein; IRF7, interferon regulatory factor 7; ZBP1, Z-DNA-binding protein 1.

precise regulatory network in inflammatory responses. Through their concentration gradients, tissue-specific distribution, and synergistic effects with other factors, this network profoundly influences the decision-making logic of cell death patterns (e.g., apoptosis, necroptosis, pyroptosis), thereby shaping the progression and outcome of inflammation. Concentration-dependent regulation is a core feature of this network. Taking TNF-α as an example, its signal output exhibits significant concentration dependence. Under low concentration conditions, TNF-α primarily activates the NF-κB pathway to promote cell survival and inflammatory gene expression [216]. However, when concentrations increase or when specific signal interference is present, such as when caspase-8 activity is inhibited, TNF-α can effectively induce caspase-8-dependent apoptosis or RIPK1/RIPK3/MLKL-mediated necroptosis [217]. Among these, the phosphorylation status of RIPK1 is a critical molecular switch for sensing signal intensity and determining cell fate towards survival, apoptosis, or necroptosis

[91]. Synergism is another important mechanism. IFN-γ itself can regulate immune cell function through the JAK-STAT pathway, such as the enhancement of macrophage bactericidal activity, dendritic cell (DC) antigen presentation, and $CD8^+$ T cell cytotoxicity [218]. However, its core function lies in significantly "sensitizing" cells to other death signals. IFN-γ can greatly enhance cellular sensitivity to factors like TNF-α, collectively promoting the occurrence of apoptosis or necroptosis. This synergistic effect is particularly prominent under pathological conditions; in the cytokine storm induced by SARS-CoV-2 infection, the synergy of TNF-α and IFN-γ can induce PANoptosis, leading to severe tissue damage [219]. The tissue microenvironment, in turn, provides a third dimension of regulation. The ultimate cellular response to TNF-α and IFN-γ signals is not isolated but profoundly depends on the surrounding tissue environment. The influence of the tissue microenvironment on cell death patterns has its molecular basis deeply rooted in the response of subcellular structures. Mitochondria, as primary sensors of microenvironmental stress (e.g., hypoxia, nutrient deprivation), have their functional status (energy production, ROS levels, membrane integrity) directly determining whether cells undergo apoptosis or trigger a strong inflammatory response [220]. The endoplasmic reticulum, in turn, senses protein synthesis burden and folding stress in the microenvironment, and the intensity and duration of its stress response regulate the cell death threshold and inflammatory cytokine secretion [221]. The nucleus integrates signals from the microenvironment (e.g., genotoxic stress), influencing the local inflammatory environment by activating p53-mediated apoptosis or regulating inflammation-related gene networks [222]. More importantly, the ubiquitination modification system, as a highly plastic regulatory layer, can dynamically modify key proteins (e.g., RIPK1, NF-κB pathway components) based on microenvironmental cues, finely tuning cellular sensitivity to death signals and the intensity and nature of the inflammatory response [223]. Therefore, understanding the functional changes in mitochondria, endoplasmic reticulum, and nucleus, as well as the dynamic regulation of the ubiquitination network, is key to elucidating how the tissue microenvironment shapes cell death patterns and their accompanying inflammatory responses. Therefore, understanding how inflammatory signals, particularly TNF-α and IFN-γ, precisely regulate cell death patterns through concentration gradients, synergistic effects, and integration with the tissue microenvironment, is crucial for uncovering the pathogenesis of inflammation-related diseases and developing targeted intervention strategies. This multilevel regulatory network enables a tight coupling between cell death and inflammation. It is precisely this bidirectional interaction mechanism that provides the molecular basis for the formation of the death-inflammation feedback loop.

In the pathological processes of various diseases, the death-inflammation feedback loop plays a core driving role, characterized by a self-amplifying cycle formed between cell death events and inflammatory responses. Among these, the pyroptosis-NLRP3 cascade amplification loop, the NETosis-IFN-α reciprocal promotion loop, and the PANoptosis integrated cell death pathway are key components of this circuit and also potential therapeutic targets. The pyroptosis-NLRP3 positive feedback loop begins with NLRP3 inflammasome-mediated caspase-1 activation, which cleaves GSDMD protein to form plasma membrane pores, executing the pyroptosis process and releasing pro-inflammatory factors such as IL-1β and IL-18 [224]. A critical turning point is that the released DAMPs can trans-activate NLRP3 inflammasomes in adjacent cells, thereby forming a "pyroptosis-DAMPs release-NLRP3 re-activation" cyclic amplification pathway. This loop is particularly prominent in acute inflammatory conditions such as sepsis and gout. Targeted intervention strategies, such as the NLRP3 inhibitor MCC950 or the caspase-1 inhibitor VX-765, can inhibit the cascade reaction by blocking the initial steps of the loop [225]. The NETosis-IFN-α reciprocal loop demonstrates an intercellular synergistic effect. Neutrophils release NETs via NETosis, thereby activating plasmacytoid DCs through PRRs such as TLR9 and inducing abundant production of IFN-α. Notably, IFN-α upregulates the expression of PAD4 via the JAK-STAT signaling pathway, promoting histone citrullination and chromatin decondensation, thereby enhancing the propensity for neutrophil NETosis and forming an intercellular signaling closed loop [226]. This loop plays a central role in autoimmune diseases such as SLE, and DNase I degradation of NETs or JAK inhibitors blocking IFN signaling can effectively break the cycle [227]. The PANoptosis integrative death pathway represents a higher-level regulatory mode. Mediated by sensor proteins such as ZBP1 and AIM2, the assembly of adaptor molecules such as RIPK1 forms the PANoptosome multiprotein complex platform, which is unique in its ability to simultaneously activate caspase-1, caspase-8, and RIPK3 pathways [228]. This synergistic effect leads to a mixed death phenotype and the release of a large amount of DAMPs, inducing a systemic inflammatory storm. In severe COVID-19 infection and hemophagocytic syndrome, targeting key nodes such as ZBP1 can disrupt complex assembly, providing new insights for curbing lethal inflammation [229].

5 DYSREGULATED INTERACTION BETWEEN CELL DEATH AND INFLAMMATION IN DISEASE CONTEXTS

Under physiological conditions, the interaction between cell death and inflammation constitutes a delicate balance: apoptotic cells are promptly cleared and release anti-inflammatory factors to promote tissue repair, whereas controlled pro-inflammatory cell death, such as pyroptosis, assists in pathogen clearance. However, when genetic susceptibility, pathogen invasion, or metabolic disorders disrupt this balance, characteristic dysregulation of this interaction is triggered. In autoimmune diseases, defective clearance of dead cells leads to persistent exposure of nuclear antigens, such as citrullinated histones. This exposure, via cGAS-STING or TLR9 pathways, induces a type

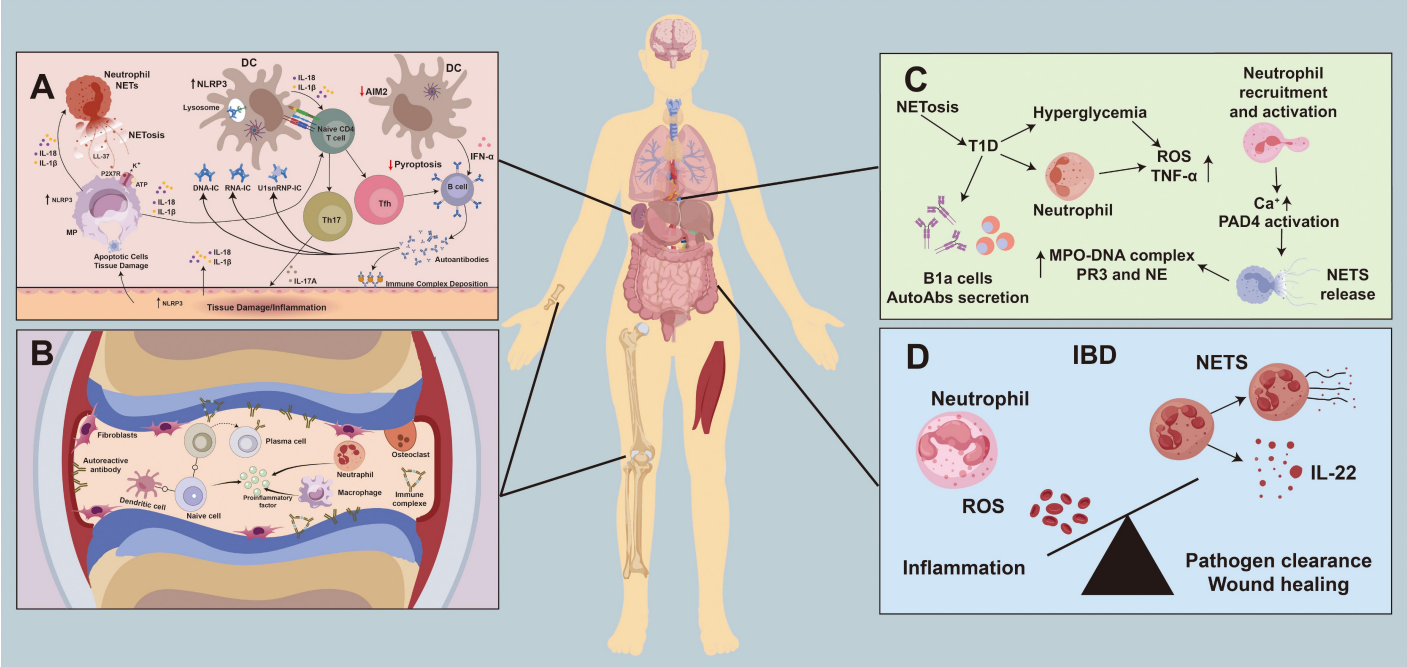

Figure 6. The multifaceted roles of neutrophils in tissue homeostasis and disease pathogenesis. Schematic summary of neutrophil functions across distinct physiological and pathological contexts. (A) Neutrophil interaction with adaptive immunity in tissue damage. Neutrophils engage in bidirectional crosstalk with DCs and Th cells, undergoing NETosis and releasing cytokines (e.g., IL-1β, IL-18 via pyroptosis), which can amplify inflammatory responses and influence T cell polarization. (B) Neutrophil involvement in the arthritic joint microenvironment. Neutrophils are present alongside plasma cells, fibroblasts, and immune complexes, contributing to chronic inflammation and tissue destruction through the release of proteases and ROS. (C) Mechanism of NETosis in T1D. In a hyperglycemic milieu, neutrophils are activated by ROS and TNF-α, leading to the release of NETs containing MPO-DNA complexes. This process is implicated in β-cell damage and the propagation of autoimmunity. (D) Dual role of neutrophils in IBD. Neutrophils exhibit a Janus-faced function: they drive tissue damage through robust ROS production but also contribute to mucosal healing and host defense via the secretion of IL-22, highlighting their context-dependent role in resolving inflammation. NET, neutrophil extracellular trap; IL-1β, interleukin-1β; IL-37, interleukin-37; P2X7R, P2X purinoceptor 7/P2X7 receptor; ATP, adenosine triphosphate; NLRP3, NLR family pyrin domain containing 3; MP, macrophage; DC, dendritic cell; DNA-IC, DNA-containing immune complex; RNA-IC, RNA-containing immune complex; U1snRNP-1C, U1 small nuclear ribonucleoprotein-immune complex; IL-17A, interleukin-17A; Th, T helper cell; Th17, T helper 17 cell; AIM2, absent in melanoma 2; IFN-α, interferon-alpha; T1D, type 1 diabetes; ROS, reactive oxygen species; TNF-α, tumor necrosis factor-alpha; B1a cells, B-1a lymphocytes; autoAbs secretion, autoantibodies secretion; MPO-DNA, myeloperoxidase-DNA complex; PR3, proteinase 3; NE, neutrophil elastase; PAD4, peptidylarginine deiminase 4; IBD, inflammatory bowel disease; IL-22, interleukin-22; IL-18, interleukin-18.

I interferon storm, driving autoimmune attacks. In infectious diseases, pathogens directly manipulate cell death programs, inducing necroptosis or PANoptosis cascades, which release excessive DAMPs and ignite systemic inflammatory responses. In metabolic diseases, nutritional stress can shift protective apoptosis towards pro-inflammatory pyroptosis or ferroptosis, forming a vicious cycle of chronic inflammation and metabolic dysfunction. However, the perioperative period—characterized by surgical stress, ischemia-reperfusion injury, and anesthetic exposure—can profoundly disrupt this equilibrium, accelerating or unmasking subclinical pathologies. This article will systematically analyze the unique mechanisms of cell death-inflammation axis dysregulation in these three types of diseases and discuss their specific implications for perioperative risk stratification, organ protection, and the development of targeted intervention strategies tailored to surgical patients.

5.1 Autoimmune diseases

Autoimmune diseases are conditions in which the immune system attacks the body's own tissues, leading to chronic inflammation, tissue damage, and systemic dysfunction. Examples of autoimmune diseases include RA, SLE, type 1 diabetes mellitus (T1DM), and IBD, as shown in **Figure 6**. These diseases affect approximately 10% of the global population, imposing a significant health and economic burden worldwide [230]. The pathogenesis of autoimmune diseases is complex, involving not only genetic susceptibility but also environmental triggers and immune dysregulation. Increasing evidence indicates that dysregulation of the cell death-inflammation axis leads to autoimmune diseases. In SLE, the over-formation of NETs and impaired apoptotic cell clearance lead to the release of autoantigens, activating B cells to produce autoantibodies, which form immune complexes and exacerbate inflammatory

responses [231]. In RA, GSDMD-mediated pyroptosis and the overexpression of IL-1β promote the formation of a synovial inflammatory microenvironment, leading to joint damage [211]. In T1DM, the death of pancreatic beta cells releases autoantigens, activating autoreactive T cells, leading to immune system attack on islet cells [232]. In IBD, ferroptosis of intestinal epithelial cells disrupts the intestinal barrier and exacerbates intestinal inflammatory responses [233]. Patients with autoimmune diseases present unique challenges in the perioperative period, as surgical stress can potentially exacerbate underlying dysregulated immunity and inflammation, increasing the risk of postoperative flares and complications. Therefore, the complex interplay between cell death and inflammation plays a crucial role in the pathogenesis and progression of various autoimmune diseases, and the regulation targeting this axis may offer new strategies not only for the treatment of autoimmune diseases but also for perioperative management to improve surgical outcomes in this vulnerable population.

SLE is a complex autoimmune disease, whose pathological essence lies in a vicious cycle formed between cell death and inflammatory responses [234]. The dysregulation of this complex network begins with abnormal regulation of the apoptotic process. In patients, impaired clearance of apoptotic cells leads to a large accumulation of uncleared cellular debris in tissues. Autoantigens released from these fragments (e.g., DNA, histones) activate the TLR pathway, stimulating plasmacytoid DCs to produce excessive IFN-I, which in turn triggers the differentiation of autoreactive B cells and the production of autoantibodies such as anti-dsDNA antibodies [235, 236]. Notably, this process is intertwined with the abnormal activation of inflammatory cell death pathways. Necroptosis mediates cell membrane rupture via the RIPK3/MLKL pathway, while pyroptosis relies on GSDMD to form plasma membrane pores; both jointly promote the release of pro-inflammatory factors such as IL-1β and IL-18, directly exacerbating tissue inflammation [237]. In the renal tissue of lupus nephritis patients, the abnormally high expression of these inflammatory death markers has been confirmed to be associated with organ damage [238]. Simultaneously, ferroptosis and NETosis play synergistic roles in the immunopathology of SLE [239]. Ferroptosis-driven lipid peroxidation not only directly damages cells but also activates neutrophils and macrophages through its released oxidative stress products, amplifying the inflammatory cascade. Studies have found that inhibiting ferroptosis can alleviate disease progression in SLE mouse models, indicating that ferroptosis plays an important role in the pathogenesis of SLE [238]. Meanwhile, NETs excessively released by neutrophils, as DNA-histone-rich reticular structures, serve both as sources of autoantigens and as participants in immune complex formation. After these complexes deposit in target organs such as the kidneys and skin, they ultimately lead to characteristic lesions like glomerulonephritis and malar rash through complement activation and Fc receptor-mediated inflammatory responses [240]. The latest research further reveals that immunometabolic dysregulation (such as glycolysis/oxidative phosphorylation imbalance) in SLE patients exhibits cross-regulation with cell death pathways [241]. Metabolic disorders can alter immune cell death thresholds, such as by enhancing neutrophil NETosis propensity or reducing macrophage apoptotic clearance capacity, thereby forming a "metabolism-death-inflammation" positive feedback loop. Based on these mechanisms, novel therapeutic strategies focus on multi-target intervention. DNase I degradation of NETs can reduce autoantigen exposure [242]. Ferroptosis inhibitors (such as Liproxstatin-1) can alleviate oxidative stress damage [243]. Small molecule inhibitors targeting RIPK1 or caspase-1 can block inflammatory cell death pathways. These interventions have shown a dual benefit of alleviating renal inflammation and reducing autoantibody levels in preclinical models.

As a chronic inflammatory disease, RA has a pathological process closely related to the complex interplay between cell death and inflammatory responses. Dysregulation of various cell death pathways, including apoptosis, necroptosis, pyroptosis, and ferroptosis, collectively drives characteristic lesions such as synovial inflammation, cartilage destruction, and bone erosion [244]. In the pathogenesis of RA, inhibition of apoptosis in synovial cells leads to abnormal synovial proliferation and persistent inflammation. Necroptosis, on the other hand, releases intracellular contents through cell membrane rupture, amplifying the inflammatory cascade. Notably, IFN-γ can participate in the regulation of inflammatory death by modulating necroptosis [245]. Pyroptosis, as a highly inflammatory form of cell death mediated by caspase-1 activation, promotes the release of pro-inflammatory factors such as IL-1β and IL-18. Among these, macrophage pyroptosis has been confirmed to promote RA progression through mechanisms such as DNA polymerase β deficiency [246]. Ferroptosis is characterized by iron-dependent lipid peroxidation and ROS accumulation, and its association with ROS-dependent apoptosis suggests new pathological dimensions [247]. Dysregulation of these cell death pathways collectively leads to the release of pro-inflammatory mediators, forming a "death-inflammation" vicious cycle. The inflammatory response plays a central role in RA; key pro-inflammatory factors such as TNF-α, IL-1β, and IL-6 directly promote cartilage degradation and bone erosion by activating synovial fibroblasts (FLS) and immune cells [248]. T cells, B cells, macrophages, and FLS constitute a complex immune network. T cells drive pathogenesis via cytokine-dependent and independent mechanisms, while FLS mediate joint destruction by secreting matrix metalloproteinases [249, 250]. Non-coding RNAs (e.g., miR-21) play an important role in inflammation regulation by modulating the Th17/Treg balance and STAT3 signaling pathway [251]. This interplay between cell death and inflammation forms a self-reinforcing axis: dysregulation of death pathways releases pro-inflammatory mediators, and activated immune cells in turn further trigger death programs, ultimately leading to irreversible joint damage.

The core pathological feature of T1DM is the progressive destruction of pancreatic β-cells, a process driven by a vicious cycle between cell death and inflammatory responses [252]. When β-cells die through pathways such as apoptosis, necroptosis, or pyroptosis, the released intracellular antigens act as DAMPs and are recognized by DCs and macrophages, activating the NLRP3 inflammasome pathway and promoting the release of pro-inflammatory factors such as IL-1β and IL-18 [253]. This inflammatory microenvironment not only directly damages β-cell function and inhibits insulin secretion, but also activates autoreactive T and B cells, leading to the production of autoantibodies against β-cell antigens and thus forming a "death-inflammation-autoimmunity" positive feedback loop. Notably, viral infections (e.g., SARS-CoV-2) can exacerbate this process through molecular mimicry mechanisms or direct β-cell damage, while lipid metabolism disorders and miRNA expression dysregulation (e.g., miR-21-mediated gene silencing) participate in disease progression by regulating immune cell function [254, 255]. In the immunopathological network of T1DM, $CD8^+$ T cells directly kill β-cells via the perforin-granzyme pathway, while macrophages amplify the inflammatory cascade by releasing pro-inflammatory mediators and presenting antigens [256]. Autoantibodies produced by B cells not only activate the complement system but also mediate antibody-dependent cell-mediated cytotoxicity, collectively leading to a reduction in β-cell mass [257]. This multicellular collaborative immune attack ultimately triggers metabolic collapse characterized by absolute insulin deficiency.

The pathological essence of IBD lies in the imbalance of intestinal epithelial cell homeostasis, with its core driving factor being a vicious cycle between cell death pathways and inflammatory responses [258]. When programmed cell death pathways such as apoptosis, necroptosis, and pyroptosis are dysregulated, the integrity of the intestinal barrier is compromised. Overactivated necroptosis mediates cell membrane rupture via the RIPK3/MLKL pathway, releasing a large number of pro-inflammatory factors such as IL-1β and TNF-α [259]. Conversely, insufficient apoptotic function leads to the retention of damaged cells, continuously activating innate immune responses. Notably, Gasdermin family protein-mediated pyroptosis is particularly crucial in IBD. The plasma membrane pores formed by caspase cleavage of GSDMD not only promote the maturation and release of IL-1β but also provide an invasion channel for gut microbiota and their metabolites (e.g., short-chain fatty acids), exacerbating mucosal immune dysregulation [260, 261]. This death-inflammation interaction forms a self-reinforcing axis. DAMPs released by cell death activate macrophages and DCs, amplifying inflammatory signals via the NLRP3 inflammasome [262]. In turn, cytokines such as TNF-α and IL-6 induce epithelial cell death via the NF-κB/MAPK pathway. Gut microbiota dysbiosis plays an important role in this process. Overproliferation of Proteobacteria directly damages epithelial tight junctions, while their metabolic products affect the necroptosis threshold by regulating RIPK1 phosphorylation status. Genetic factors (e.g., NOD2 mutations) further promote the accumulation of intracellular PAMPs by weakening autophagic clearance capacity, forming a "microbiota-gene-death" triad that drives an inflammatory storm [263].

Beyond elucidating pathogenesis, understanding these dysregulated pathways informs perioperative risk stratification for patients with autoimmune conditions. For instance, elevated levels of circulating cell-free DNA, potentially originating from NETs, may serve as a biomarker for heightened tissue injury and immune activation, signaling an increased risk for postoperative inflammatory complications. Similarly, specific autoantibodies, such as anti-dsDNA in SLE, not only serve as diagnostic markers but may also correlate with the degree of immune dysregulation and organ vulnerability under surgical stress. This knowledge enables a more nuanced preoperative assessment, moving beyond the disease label to gauge the individual's inflammatory burden and potential for exacerbation. Consequently, perioperative management can be tailored—for example, by considering targeted anti-inflammatory prophylaxis in a patient with high NETosis biomarkers or by adjusting immunosuppressive regimens based on specific cell death pathway activities to mitigate the risk of a postoperative flare.

5.2 Infectious diseases

Sepsis is life-threatening organ dysfunction caused by a dysregulated host response to infection, and its pathogenesis is closely related to the complex relationship between cell death and inflammation. In early sepsis, PAMPs and DAMPs are released, activating PRRs such as TLRs and NLRs. This activation triggers the production of a large number of pro-inflammatory cytokines and chemokines, leading to an excessive inflammatory response [264]. This excessive activation causes widespread tissue damage and vascular leakage [265]. In late sepsis, persistent cell death and chronic inflammation impair immune cell function, leading to immunosuppression. Lymphocyte apoptosis and monocyte exhaustion reduce the body's ability to clear infection, ultimately leading to secondary infections and multiple organ failure [266]. Postoperative sepsis is a major cause of morbidity and mortality, in which the added insults of surgery and anesthesia can amplify these dysregulated pathways. Dysregulation of the cell death-inflammation axis is a key driver of the complex and dynamic immune response in sepsis, as shown in **Figure 7**. Therefore, targeted interventions to restore immune homeostasis are crucial, particularly in the high-stakes perioperative setting.

In the initial phase of sepsis, PAMPs and DAMPs are released from damaged cells, triggering the activation of innate immune cells. Activation of PRRs initiates downstream signaling pathways, leading to the massive production of pro-inflammatory cytokines. These cytokines play a crucial role in the inflammatory response, recruiting immune cells to the site of infection

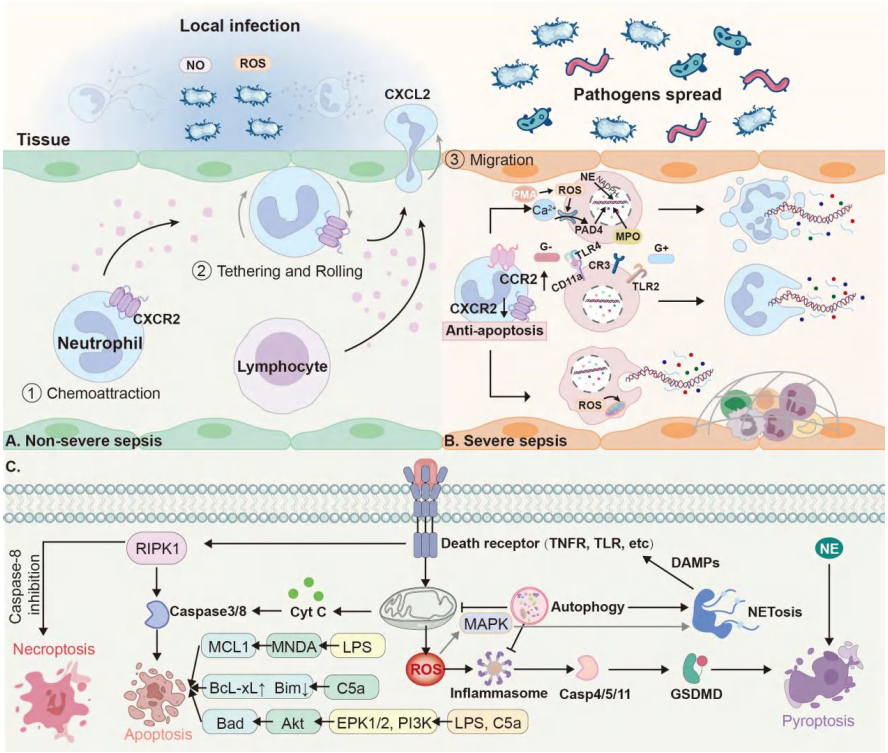

Figure 7. Dysregulated neutrophil responses determine pathological progression from localized infection to severe sepsis. Schematic model contrasting neutrophil dynamics in controlled versus dysregulated immune responses during sepsis. (A) Non-severe sepsis (controlled response). A local infection initiates a coordinated immune response: (1) Chemoattraction: Neutrophils are recruited to the site by chemokines (e.g., CXCL2). (2) Tethering and Rolling: Neutrophils adhere to and roll on the endothelium. (3) Migration: Neutrophils transmigrate into the tissue to clear pathogens, a process potentially aided by lymphocytes. This controlled response prevents systemic pathogen spread. (B) Severe sepsis (dysregulated response). Systemic pathogen dissemination leads to a hyperinflammatory state. Neutrophils exhibit enhanced migration but also exhibit resistance to apoptosis, promoted by survival signals such as increased MCL-1 and Bcl-xL, and activation of pro-survival pathways (e.g., ERK1/2, PI3K/Akt). This prolongs neutrophil lifespan, contributing to collateral tissue damage through excessive release of ROS and proteases like NE. (C) Orchestration of neutrophil cell death pathways. The panel details how specific stimuli (e.g., LPS, C5a, cytokines, DAMPs) and molecular interactions (e.g., caspase-8 inhibition) regulate the balance between distinct neutrophil death modalities, including apoptosis, necroptosis, pyroptosis, and NETosis. This balance critically influences the inflammatory outcome and disease severity. This figure integrates spatial, functional, and molecular perspectives to elucidate how neutrophil fate decisions drive the transition from a protective immune response to a pathological state in sepsis. NO, nitric oxide; ROS, reactive oxygen species; CXCL2, C-X-C motif chemokine ligand 2; CXCR2, C-X-C chemokine receptor type 2; RIPK1, receptor-interacting protein kinase 1; Cyt c, cytochrome c; MCL-1, myeloid cell leukemia 1; MNDA, myeloid cell nuclear differentiation antigen; LPS, lipopolysaccharide; Bad, BCL2-associated agonist of cell death; Akt, protein kinase B; ERK1/2, extracellular signal-regulated kinase 1/2; PI3K, phosphoinositide 3-kinase; C5a, complement component 5a; TNFR, tumor necrosis factor receptor; TLR, Toll-like receptor; DAMPs, damage-associated molecular patterns; MAPK, mitogen-activated protein kinase; Casp4/5/11, caspase-4/5/11; GSDMD, gasdermin D; Bcl-xL, B-cell lymphoma-extra large; NE, neutrophil elastase; NADPH, nicotinamide adenine dinucleotide phosphate; PMA, phorbol 12-myristate 13-acetate; PAD4, peptidylarginine deiminase 4; MPO, myeloperoxidase; CCR2, C-C chemokine receptor type 2; CD11a, cluster of differentiation 11a; TLR4, Toll-like receptor 4; CR3, complement receptor 3; TLR2, Toll-like receptor 2; Bim, BCL-2 interacting mediator of cell death.

and activating other immune cells. However, the excessive release of pro-inflammatory cytokines can lead to SIRS, characterized by fever, tachycardia, tachypnea, and leukocytosis [267]. SIRS may lead to increased vascular permeability, vasodilation, and microthrombus formation, thereby causing tissue hypoxia and organ dysfunction. Pro-inflammatory cytokines can damage endothelial cells, leading to vascular leakage and edema [268]. Endothelial damage also activates the coagulation cascade, leading to thrombus formation in microvessels, further impairing organ perfusion. Furthermore, neutrophils, major innate immune cellS, are massively recruited to the site of infection in early sepsis [269]. Activated neutrophils release proteolytic enzymes and ROS, which further damage tissues. Neutrophils can also undergo NETosis to capture and kill pathogens. However, excessive NETs formation can lead to inflammation and tissue damage.

Although early sepsis is characterized by hyperinflammation, the late stage typically involves an immunosuppressed state. This immunoparalysis renders patients susceptible to secondary infections and persistent organ dysfunction. Sepsis-induced immunosuppression is caused by multiple mechanisms, including immune cell apoptosis, immune cell exhaustion, and the expansion of suppressive immune cells [270]. Apoptosis, or programmed cell death, is an important cause of immune cell depletion in sepsis. T cells, B cells, and DCs undergo apoptosis, reducing the magnitude of the immune response [271]. Pro-inflammatory cytokines, such as TNF-α and IL-1β, and death receptor ligands, such as FasL, are involved in inducing immune cell apoptosis. Immune cell exhaustion also contributes to immunosuppression in sepsis [272]. Prolonged immune activation can lead to immune cell exhaustion, resulting in impaired function and reduced proliferative capacity. Exhausted T cells express inhibitory receptors, such as PD-1, a receptor that suppresses their activity [273]. Tregs and myeloid-derived suppressor cells are suppressive immune cells that inhibit immune responses. In sepsis, Tregs and myeloid-derived suppressor cells expand, further suppressing immune responses and promoting pathogen persistence [274]. Another important aspect of immune cell

dysfunction in sepsis is impaired antigen presentation. APCs, such as DCs and macrophages, play a crucial role in initiating adaptive immune responses [275]. In sepsis, APC function is impaired, leading to reduced antigen presentation and decreased T cell activation. Furthermore, sepsis leads to polarization of cytokine production, characterized by decreased pro-inflammatory cytokines and increased anti-inflammatory cytokines [276]. This cytokine polarization contributes to an immunosuppressive state and impairs the ability to clear infections.

Cell death plays a crucial role in the pathophysiology of sepsis, with several types of cell death involved, including apoptosis, necroptosis, pyroptosis, and autophagy. Apoptosis is a type of programmed cell death, characterized by cell shrinkage, chromatin condensation, and the formation of apoptotic bodies. Apoptosis is generally considered immune silent, as it does not release inflammatory contents [277]. However, in sepsis, excessive apoptosis can lead to immune cell depletion and promote immunosuppression. Necroptosis is mediated by the activation of RIPK1 and RIPK3. In contrast to apoptosis, necroptosis leads to the release of inflammatory mediators, thereby exacerbating the inflammatory response [1]. Pyroptosis is a form of inflammatory cell death, mediated by inflammasome activation and subsequent GSDMD cleavage. Inflammasomes are cytoplasmic multiprotein complexes that activate caspase-1, which cleaves GSDMD, leading to pore formation in the cell membrane and the release of pro-inflammatory cytokines, such as IL-1β and IL-18. Pyroptosis plays a crucial role in the pathophysiology of sepsis, contributing to the inflammatory response and organ dysfunction [278]. Autophagy is a cellular process involving the degradation and recycling of cellular components. Autophagy can play a role in cell survival and cell death, depending on the situation. In sepsis, autophagy has been shown to promote cell survival by clearing damaged organelles and protein aggregates [279]. However, excessive autophagy can lead to autophagic cell death, which is a form of cell death that can aggravate sepsis by disrupting cellular homeostasis and potentially exacerbating the inflammatory response.

Given that the cell death-inflammation axis plays a crucial role in the pathophysiology of sepsis, therapeutic strategies targeting this axis hold promise for improving the prognosis of sepsis patients. These strategies include inhibiting inflammation, modulating cell death pathways, and restoring immune function. Strategies for inhibiting inflammation include the use of anti-inflammatory drugs, such as corticosteroids and TNF-α inhibitors [280]. However, these drugs have limited effectiveness in sepsis and are associated with significant side effects [281]. Strategies to modulate cell death pathways include inhibiting apoptosis, necroptosis, and pyroptosis. Caspase inhibitors can block apoptosis and have been shown to exert protective effects in preclinical studies [282]. RIPK1 inhibitors can block necroptosis and are currently being developed for the treatment of various inflammatory diseases. Strategies to restore immune function include the use of immunostimulants, such as IFN-γ and granulocyte-macrophage colony-stimulating factor [274]. These cytokines can enhance immune cell function and improve pathogen clearance. Additionally, mesenchymal stem cells have been shown to have immunomodulatory effects in sepsis [283]. Mesenchymal stem cells can inhibit the JAK-STAT signaling pathway and regulate Th cells, thereby alleviating the cytokine storm in sepsis.

The principles of risk stratification and targeted intervention are equally critical in the context of perioperative infections and sepsis. The preoperative identification of patients at high risk for infectious complications or sepsis progression can leverage biomarkers of immune status and cellular stress. For example, profiling markers of innate immune activation, such as components of NETs or DAMPs, might identify patients with a subclinical hyperinflammatory state who are primed for an exaggerated response to surgical trauma. Furthermore, the recognition that surgical stress itself can potentiate specific cell death pathways, such as NLRP3 inflammasome-mediated pyroptosis, connects the risk of postoperative sepsis to fundamental mechanisms of tissue damage. In a patient with signs of metabolic stress (e.g., elevated oxidative stress markers), the vulnerability to ferroptosis in critical tissues like the intestinal epithelium or liver could be increased, thereby compromising barrier function and facilitating bacterial translocation. Thus, preoperative optimization might include strategies to bolster cellular resilience, such as antioxidant support in high-risk patients, while postoperative monitoring could focus on biomarkers reflective of specific death pathways (e.g., GSDMD for pyroptosis) to guide timely and mechanism-directed interventions like IL-1 blockade, moving sepsis management towards a more personalized and preemptive model.

5.3 Metabolic diseases

Metabolic diseases, such as non-alcoholic steatohepatitis (NASH) and atherosclerosis, are closely related to the complex relationship between cell death and inflammation. Patients with these conditions are at significantly increased risk for perioperative complications due to their underlying metabolic and inflammatory instability, which can be severely tested by the physiologic stress of surgery. NASH is a chronic liver disease associated with metabolic dysfunction, characterized by hepatic fat accumulation, inflammation, hepatocyte injury, and fibrosis [284]. The pathological core of NASH lies in the "death-inflammation" vicious cycle triggered by lipid metabolism disorder. When lipids excessively accumulate within hepatocytes (hepatic steatosis), toxic lipids such as saturated fatty acids induce endoplasmic reticulum stress and mitochondrial dysfunction, leading to explosive production of ROS [285]. This metabolic stress causes hepatocytes to undergo apoptosis, necroptosis, and GSDMD-mediated pyroptosis, releasing DAMPs such as HMGB1 and ATP. These DAMPs, by activating TLRs on hepatic Kupffer cells and infiltrating macrophages, initiate the NLRP3 inflammasome signaling cascade, driving a

storm of pro-inflammatory factors such as TNF-α and IL-1β [286]. Notably, a chronic inflammatory environment further disrupts metabolic homeostasis. TNF-α exacerbates insulin resistance by inhibiting insulin receptor substrate phosphorylation, while IL-1β directly inhibits fatty acid β-oxidation, forming a positive feedback loop of "lipid accumulation-cell death-inflammation-metabolic dysfunction" [287]. Persistent inflammation activates hepatic stellate cells (HSCs), transforming them into myofibroblasts, which promote the progression of liver fibrosis by secreting ECM components such as type I collagen [288]. In this process, gut microbiota dysbiosis plays a synergistic role. Gut microbiota dysregulation leads to LPS translocation, which, after entering the liver via the portal vein, promotes Kupffer cell activation through TLR4. Meanwhile, reduced short-chain fatty acids weaken the protective function of the intestinal barrier [289].

Atherosclerosis is another chronic inflammatory disease in which lipids accumulate within the arterial walls, leading to plaque formation, which can ultimately result in cardiovascular events [290]. When metabolic diseases such as obesity and diabetes trigger lipid metabolism dysregulation, they can directly induce various forms of cell death, including pyroptosis, apoptosis, and necrosis. These dying cells release a large amount of DAMPs, which, after binding with PRRs, activate downstream inflammatory pathways. Activation of inflammatory responses further exacerbates vascular endothelial damage, increases vascular permeability, and promotes lipid deposition within the arterial wall [291]. Monocytes are recruited to the vascular wall, where they differentiate into macrophages and phagocytose oxidized low-density lipoprotein, forming foam cells [292]. Excessive accumulation of foam cells ultimately leads to cell death, forming a necrotic core. This releases more DAMPs, further exacerbating the inflammatory response and creating a vicious cycle. Furthermore, the inflammatory environment also promotes the proliferation and migration of vascular smooth muscle cells, alters the composition of the ECM, leading to the formation and instability of atherosclerotic plaques [293]. This dysregulation of the axis composed of cell death and inflammation not only directly drives the initiation and progression of atherosclerosis but also causes low-grade inflammation systemically, exacerbating characteristics of metabolic diseases such as insulin resistance. This persistent, low-grade inflammatory activation and plaque instability critically underpin the increased incidence of major adverse cardiovascular events, such as myocardial infarction, in the perioperative period. The physiological stressors of surgery—including catecholamine surges, hemodynamic instability, and a hypercoagulable state—can disrupt vulnerable, inflammation-rich atherosclerotic plaques. This disruption exposes pro-thrombotic material, triggering acute thrombosis and vessel occlusion, especially in plaques where ongoing inflammatory cell death (e.g., macrophage necroptosis) has already weakened the fibrous cap.

Although NASH and atherosclerosis have distinct target organs, they share the core pathological paradigm of "lipid metabolism disorder-triggered cell death-inflammation vicious cycle". At the molecular level, both exhibit saturated fatty acid-induced endoplasmic reticulum stress and mitochondrial dysfunction, events that activate multiple death pathways through ROS burst, thereby releasing DAMPs such as HMGB1/ATP. These danger signals, via the TLR4/NLRP3 axis, amplify the inflammatory cascade, forming a "cell death-inflammation-exacerbation" self-reinforcing loop. Meanwhile, organ-specific effects also shape differences in disease phenotypes. In NASH, HSCs transform into myofibroblasts, driving collagen deposition. In contrast, in atherosclerosis, foam cell necrosis determines plaque stability. This difference stems from microenvironmental specialization. The liver amplifies inflammation via the gut-liver axis (LPS translocation), while the vascular system accelerates endothelial damage through blood flow in conjunction with oxidized low-density lipoprotein deposition. From a systems biology perspective, metabolic diseases are essentially a multi-organ dialogue triggered by the dysregulation of the "death-inflammation axis". Adipose tissue inflammation exacerbates the liver burden through lipid overflow, and acute phase proteins released by the liver, in turn, promote vascular inflammation, while insulin deficiency caused by pancreatic β-cell apoptosis further amplifies systemic metabolic disorders. This cross-organ interaction highlights the complexity of targeted therapy — single-organ intervention may struggle to break the systemic vicious cycle. Consequently, patients with pre-existing NASH present a significantly elevated risk for postoperative hepatic dysfunction. The underlying susceptibility stems from the chronic inflammatory milieu and metabolic inflexibility, which predispose hepatocytes to exacerbated cell death (particularly ferroptosis and necroptosis) when confronted with the obligatory stressors of major surgery, such as transient hepatic ischemia-reperfusion, oxidative stress from anesthetic metabolism, and hemodynamic fluctuations. This compromised hepatic reserve can readily culminate in clinically significant postoperative liver injury. Therefore, future strategies need to focus on multi-system levels, rather than being limited to a single system. This systems approach is especially relevant for perioperative medicine, where optimizing the metabolic and inflammatory state before surgery (prehabilitation), protecting organs during the procedure, and carefully managing recovery could significantly improve outcomes. Only through integrated interventions can we break through the current treatment bottleneck of metabolic inflammatory diseases and their impact on surgical care.

This systemic perspective is fundamental to perioperative risk modification in metabolic disease. Preoperative risk assessment must extend beyond standard metrics to include biomarkers reflective of underlying cell death and inflammatory pathways. For instance, elevated oxidative stress markers in a patient with metabolic dysfunction-associated steatotic liver disease may indicate a heightened susceptibility to ferroptosis-

driven hepatic injury during ischemia-reperfusion events common in surgery. Similarly, assessing the activity of inflammasome pathways could stratify the risk for exacerbated systemic inflammation postoperatively. The goal of prehabilitation in these patients, therefore, includes not only metabolic optimization (e.g., glycemic control) but also potentially modulating these cellular vulnerability pathways. Perioperative organ protection strategies can then be targeted: for a patient with high oxidative stress burden, anesthesia and adjunctive drugs with antioxidant properties (e.g., propofol) or consideration of specific cytoprotective agents might be favored. This approach transforms perioperative care from a reactive to a proactive and precision-based practice, where the molecular profile of a patient's metabolic disease directly informs the protective strategy to mitigate their unique risks of organ injury.

5.4 Perioperative stimuli and cell death induction

Multiple perioperative factors, including propofol anesthesia, surgical trauma, and intraoperative hypotension, constitute significant stressors that can activate complex cellular stress responses. This often leads to the induction of various forms of regulated cell death (RCD), such as apoptosis, pyroptosis, necroptosis, ferroptosis, and autophagic cell death. A nuanced understanding of how these distinct stimuli preferentially engage specific RCD pathways is crucial for developing targeted organ-protective strategies in the perioperative period.

The intravenous anesthetic propofol exerts a dual, context-dependent influence on cellular fate. Beyond its primary pharmacological action, evidence suggests propofol can modulate cell death pathways. For instance, it may upregulate specific microRNAs (e.g., miR-20b) to suppress autophagic cell death in endothelial cells subjected to hypoxia/reoxygenation stress, indicating a potential protective role [294]. Conversely, propofol may also influence the delicate balance between apoptosis and pyroptosis by modulating mitochondrial function and ROS generation, highlighting its complex interplay with cell survival and inflammatory death mechanisms.

Surgical trauma, a major perioperative insult, triggers a systemic inflammatory and neuroendocrine response that broadly activates multiple RCD pathways. Ischemia-reperfusion injury, a common consequence of surgical procedures, involves a spatial hierarchy of cell death: while the central ischemic zone typically undergoes necrosis, the surrounding penumbra is characterized by the activation of apoptosis, a non-inflammatory, orderly form of PCD [295]. Concurrently, trauma-induced metabolic stress, iron dyshomeostasis, and GSH depletion can create a cellular environment conducive to ferroptosis, driven by iron-dependent lipid peroxidation [296]. Furthermore, the inflammatory milieu and direct cellular damage can activate receptor-interacting protein kinases, leading to necroptosis, a lytic and pro-inflammatory form of death mediated by RIPK1/RIPK3/MLKL. In parallel, DAMPs released from injured tissues can activate inflammasomes, culminating in pyroptosis—a highly inflammatory death executed by gasdermin family proteins, which forms pores in the plasma membrane to release potent cytokines like IL-1β.

Intraoperative hypotension, resulting in tissue hypoperfusion and ischemia, is a direct catalyst for cellular injury and death. Organs with high metabolic demand, such as the kidneys, are particularly vulnerable. The resulting energy crisis (ATP depletion) and oxidative stress from ischemia-reperfusion can simultaneously engage several of the aforementioned RCD pathways, contributing to organ dysfunction like acute kidney injury [297].

Given this complexity, the development of specific intervention strategies is paramount. Targeting oxidative stress with antioxidants represents a foundational approach, particularly against ferroptosis. More precise pharmacological modulation is also possible: BH3 mimetics can tip the balance towards apoptosis in specific contexts, while inhibitors targeting RIPK1, RIPK3, or MLKL can block necroptosis. Similarly, inflammasome or GSDMD inhibitors hold promise for mitigating pyroptosis. For ferroptosis, iron chelators, lipophilic radical-trapping antioxidants, or GPX4-stabilizing compounds are under investigation.

In conclusion, perioperative stimuli induce a network of interconnected RCD pathways that collectively determine the extent of tissue injury. Future therapeutic efforts must move beyond broad-spectrum cytoprotection towards context-specific, pathway-targeted interventions. A deeper mechanistic understanding of how factors like propofol or surgical trauma bias cells towards particular death fates (e.g., modulating the apoptosis/pyroptosis balance) will be essential for developing next-generation, precision-based organ protection protocols in anesthesiology and perioperative medicine.

5.5 Cytoprotective modulation by anesthetic agents and techniques

The strategic selection of anesthetic agents and techniques extends beyond the provision of unconsciousness and analgesia, actively influencing patient recovery through distinct cytoprotective mechanisms. Volatile anesthetics, such as sevoflurane and desflurane, confer protection primarily via the well-established phenomena of anesthetic preconditioning and postconditioning. These effects are mediated through the activation of crucial pro-survival pathways, notably the PI3K/Akt/GSK-3β signaling axis, which inhibits apoptotic and necroptotic processes [298]. A key mitochondrial mechanism involves the opening of mitochondrial ATP-sensitive potassium channels, which stabilizes membrane potential and potently inhibits the mitochondrial permeability transition pore, a terminal event in cell death [299]. Furthermore, volatile agents modulate ROS signaling, harnessing a controlled burst for protective signaling

while mitigating excessive oxidative damage during ischemia/reperfusion injury [300].

In contrast to volatile agents, the intravenous anesthetic propofol exerts its protective effects largely through a potent antioxidant and anti-inflammatory synergy. Its efficacy is attributed to the activation of the Nrf2/HO-1 pathway, a master regulator of the cellular antioxidant response, and the direct suppression of the NLRP3 inflammasome, thereby curtailing the release of pro-inflammatory cytokines like IL-1β [301]. Propofol also demonstrates protective effects against RCD, such as inhibiting ferroptosis in cardiomyocytes, contributing to its role in neuroprotection and mitigation of systemic inflammation.

Beyond general anesthetics, regional techniques like epidural analgesia provide organ protection indirectly by fundamentally modulating the surgical stress response. Effective nociceptive blockade at the neural axis attenuates the systemic neuroendocrine stress response, leading to reduced catecholamine and cortisol release [302]. This promotes hemodynamic stability and yields a significant opioid-sparing effect, which in turn avoids opioid-related side effects such as ileus and immune suppression. Importantly, by blunting systemic inflammation and improving splanchnic perfusion, regional anesthesia helps preserve intestinal barrier integrity, reducing enterocyte apoptosis and the risk of bacterial translocation—an effect that is crucial for patient recovery [303].

The clinical implications of these mechanistic differences are substantial. In cardiac surgery, the preconditioning properties of volatile anesthetics are a strategic consideration for myocardial protection. The choice between intravenous and inhaled agents may influence neurological outcomes, given their differential impacts on neuroinflammation. Within Enhanced Recovery After Surgery protocols, regional anesthesia is foundational, accelerating gastrointestinal recovery and improving pain management while minimizing opioid use. Thus, an understanding of these cytoprotective mechanisms is integral to tailoring anesthetic plans for improved perioperative outcomes.

6 CLINICAL APPLICATION PROSPECTS

The dynamic interplay between cell death and inflammatory responses constitutes a central hub in disease pathology, and targeted intervention at its key nodes is providing new therapeutic paradigms for various intractable diseases, as shown in **Table 3**. When cells undergo apoptosis, necroptosis, or pyroptosis, the released DAMPs, by activating TLRs and inflammasomes (e.g., NLRP3), drive a storm of pro-inflammatory factors such as TNF-α and IL-1β. This process can induce ICD in the tumor microenvironment, activating DCs to present tumor antigens and enhancing cytotoxic T lymphocyte responses. However, in autoimmune diseases, the same pathway leads to tissue damage due to overactivation [211]. This dual effect highlights the precise need for targeted strategies. Caspase inhibitors can block the dysregulation of apoptosis-induced immune silencing; RIPK1/3 small molecule antagonists (e.g., Nec-1s) can inhibit the pro-inflammatory effects of necroptosis, while GSDMD inhibitors alleviate pyroptosis-related inflammatory storms by blocking plasma membrane pores [320, 321]. In clinical translation, these targets demonstrate cross-disease application potential. Taking cancer therapy as an example, ferroptosis inducers (e.g., erastin) selectively eliminate drug-resistant tumor cells by depleting GSH and produce synergistic effects with PD-1 inhibitors [98]. In autoimmune diseases such as RA and IBD, TNF-α monoclonal antibodies (infliximab) and IL-1β antagonists (canakinumab) have established clinical status. Meanwhile, an emerging NLRP3 inhibitor (MCC950) can alleviate synovial and intestinal mucosal inflammation by blocking inflammasome assembly. In the field of neurodegenerative diseases, the focus is on inhibiting microglia overactivation, and inhibitors targeting the STING pathway can reduce β-amyloid-induced neuroinflammation [322]. Meanwhile, ROS scavengers (e.g., edaravone) can delay neuronal death by protecting mitochondrial function [323].

In the field of drug development, two major strategies, drug repurposing and intelligent delivery, are leading the innovation of therapeutic paradigms. Drug repurposing significantly shortens the research and development cycle by exploring the multi-target potential of existing drugs. The hypoglycemic drug metformin has been repurposed as an anti-tumor agent, which induces apoptosis in oral squamous cell carcinoma cells by activating the AMPK/mTOR pathway [324]. The antifungal drug Terbinafine hydrochloride inhibits skin cancer growth by regulating keratin metabolism [325]. This strategy is particularly crucial in neurodegenerative diseases. New breakthroughs in the treatment of Alzheimer's disease are being made through network pharmacology analysis-driven drug repositioning, revealing common regulatory nodes for β-amyloid clearance and tau protein phosphorylation [326]. Smart delivery systems break through the limitations of traditional drug delivery through spatiotemporally precise regulation; nanocarriers based on stimulus-responsive polymers (e.g., pH-sensitive hydrogels, ROS-responsive liposomes) can achieve programmed drug release at the lesion site. In tumor therapy, the EGCG/doxorubicin co-delivery system, through tumor microenvironment-triggered drug release, synergistically induces ICD and significantly enhances the efficacy of anti-PD-1 therapy [327]. In chronic inflammatory diseases, M1 macrophage-targeted nanoparticles can selectively deliver IL-10, reversing the pro-inflammatory phenotype without inducing systemic immunosuppression [328].

The clinical translation of these therapeutic strategies relies on precise navigation by biomarkers. When cell death occurs, molecular events such as cleaved caspase-3, RIPK1 phosphorylation levels, and GSDMD pore formation translate into detectable biomarker profiles, which not only reflect the pathological

Table 3. Clinical intervention strategies targeting the cell death-inflammation axis

Target type	Representative drug(s)	Mechanism of action	Indication	Development stage	Limitations	References
NLRP3 inflammasome inhibitors	MCC950	Blocks NLRP3 ATPase domain oligomerization	NASH	Phase II	Off-target effects, hepatotoxicity	[304]
	Oltipraz	Activates Nrf2→Inhibits NLRP3 expression	Diabetic nephropathy	Preclinical	Photosensitivity rash	[305]
RIPK1 kinase inhibitors	Nec-1s (GSK'772)	Selectively inhibits RIPK1 kinase activity	Ulcerative colitis	Phase II	Infection risk	[306]
	SAR443122	Dual-target RIPK1/TBK1 inhibition	Systemic lupus erythematosus (SLE)	Phase II	Anemia	[307]
GSDMD pore blockers	Disulfiram (Repurposing)	Covalently binds GSDMD Cys191→Blocks pore formation	Sepsis	Phase III (Repurposing)	Copper accumulation toxicity	[308]
	Necrosulfonamide	Targets MLKL→Blocks necroptotic pore	Myocardial infarction	Preclinical	Poor solubility	[203]
Ferroptosis inducers	B23 (Genipin derivative)	Modulates expression of ACSL4, GPX4, FTH1→Induces ferroptosis	Triple-negative breast cancer	Preclinical	Limited experimental data	[309]
	RSL3	Covalently binds and inactivates GPX4	Castration-resistant prostate cancer	Phase I	Hepatotoxicity	[310]
Cuproptosis modulators	Tetrathiomolybdate (TTM)	Copper chelator→Reduces free copper	Breast cancer (high relapse risk)	Approved	Copper deficiency→Anemia	[311]
	Elesclomol	Copper ionophore→Targets mitochondria→Induces cuproptosis	Melanoma	Phase II	Increased adverse events when combined with paclitaxel	[312]
NETosis inhibitors	DNase I	Hydrolyzes DNA strands within NETs	Lupus nephritis (SLE)	Phase III	Immunogenicity, reduced efficacy	[313]
	GSK484	Inhibits PAD4→Blocks histone citrullination→Inhibits NET formation	Rheumatoid arthritis (RA)	Phase II	Thrombocytopenia	[314]
STING pathway antagonists	H-151	Covalently binds STING Cys91→Blocks dimerization	SLE-associated interferonopathy	Preclinical	Immunosuppression	[315]
TLR4 signaling blockers	Eritoran	Inhibits TLR4-mediated inflammation & HMGB1 signaling	Chronic liver injury & fibrosis	Animal models	Does not improve systemic insulin resistance	[316]
PANoptosome interferers	Cercosporamide	Inhibits Mnk kinase activity	Alzheimer's disease	Preclinical	Complex mechanism, potential off-target effects	[229]
Autophagy inhibitor	Hydroxychloroquine (HCQ)	Alkalizes lysosomes→Blocks autophagosome degradation	BRAF mutant melanoma	Phase II	Retinal toxicity	[317]
cGAS inhibitors	RU.521	Competitively inhibits cGAS catalytic activity	Subarachnoid hemorrhage	Preclinical	Off-target effects	[318]
S100A9 inhibitor	Paquinimod	Blocks S100A9-TLR4 interaction	HFpEF (Heart Failure w/preserved EF)	Phase II	Mechanism not fully elucidated	[319]

Note: NLRP3, NLR family pyrin domain containing 3; ATPase, adenosine triphosphatase; NASH, non-alcoholic steatohepatitis; Nrf2, nuclear factor erythroid 2-related factor 2; RIPK1, receptor-interacting protein kinase 1; Nec-1s, necrostatin-1s; TBK1, TANK-binding kinase 1; SLE, systemic lupus erythematosus; GSDMD, gasdermin D; Cys191, cysteine 191; MLKL, mixed lineage kinase domain-like pseudokinase; ACSL4, acyl-CoA synthetase long-chain family member 4; GPX4, glutathione peroxidase 4; FTH1, ferritin heavy chain 1; RSL3, RAS-selective lethal 3; TTM, tetrathiomolybdate; PAD4, peptidylarginine deiminase 4; RA, rheumatoid arthritis; STING, stimulator of interferon genes; Cys91, cysteine 91; TLR4, Toll-like receptor 4; HMGB1, high mobility group box 1; Mnk, mitogen-activated protein kinase-interacting kinase; HCQ, hydroxychloroquine; BRAF, B-Raf proto-oncogene serine/threonine-protein kinase; cGAS, cyclic GMP-AMP synthase; S100A9, S100 calcium-binding protein A9; HFpEF, heart failure with preserved ejection fraction; EF, ejection fraction.

state but also possess prognostic capabilities. For example, an elevated serum neutrophil-to-lymphocyte ratio in non-small cell lung cancer patients predicts poor prognosis, while the dynamics of the inflammatory protein network after myocardial infarction are closely related to cardiac function recovery [329, 330]. In the field of diagnosis, fecal calprotectin levels in patients with IBD are positively correlated with the degree of intestinal mucosal inflammation, serving as an alternative endoscopic assessment indicator [331]. In patients with SLE, serum anti-dsDNA antibody titers are directly associated with the risk of renal involvement [332]. At the level of treatment monitoring, synchronous analysis of PD-1/PD-L1 expression and $CD8^+$ T cell infiltration can assess the response to immunotherapy in real-time [333]. When necroptosis inhibitors are used to treat RA, changes in serum MLKL levels directly reflect the target inhibition efficiency [334]. Emerging liquid biopsy technologies further expand monitoring dimensions. Circulating tumor DNA mutation profiles track tumor clonal evolution, while exosomal miRNAs reveal the inflammatory status of the microenvironment, providing molecular navigation for dynamic adjustment of treatment strategies [335].

Despite its broad prospects, clinical translation still faces triple challenges. In the field of targeted therapy, there is an urgent need to overcome the complexity of spatiotemporal dynamic regulation. During the acute phase, precise blockade of death signaling is required, while during the chronic phase, it is necessary to reverse the fibrotic process. Meanwhile, technical bottlenecks in organ-specific delivery urgently need to be overcome, such as developing nanocarriers that can efficiently penetrate the blood-brain barrier to achieve precise drug delivery to the central nervous system. In drug development, drug repurposing strategies often trigger off-target effects due to the inherent multi-target characteristics of drugs, limiting their maximal therapeutic efficacy. Intelligent delivery systems, however, face scale-up production bottlenecks for industrial translation from laboratory to industry, restricting clinical accessibility. In the application of biomarkers, the lack of standardization remains a key obstacle, typically exemplified by the absence of unified standards for detecting the activity threshold of the pyroptosis execution protein GSDMD. Furthermore, organ cross-interference severely impacts biomarker specificity; for example, cardiac troponin often yields false-positive results in patients with renal failure, leading to clinical misjudgment. Overcoming these obstacles requires synergistic technological innovation. AI-driven multi-omics analysis predicts drug-target interactions, organ-on-a-chip models simulate death-inflammation dynamics, and CRISPR detection platforms enhance biomarker sensitivity. Closed-loop feedback intelligent delivery systems, in turn, adjust drug release rates in real-time based on biomarkers, achieving a "monitor-intervene-reassess" precision medicine closed loop. This integrated strategy will usher in a new era for the individualized treatment of cancer, autoimmune diseases, and neurodegenerative diseases.

7 DISCUSSION

The interactive network of cell death and inflammation constitutes the core axis of homeostatic regulation in the body, and its dynamic imbalance is a key driving factor in the occurrence of major diseases. The perioperative period, with its confluence of surgical trauma, anesthetic exposure, and potential ischemia-reperfusion injury, represents a critical window where this delicate balance is profoundly tested, often determining the trajectory of postoperative recovery and the risk of complications. Furthermore, the temporal dynamics of these pathways in response to acute intraoperative insults are particularly critical, implying that the timing of potential interventions relative to the surgical stressor is a key determinant of efficacy, a concept central to perioperative medicine. Different cell death modalities shape unique inflammatory responses by releasing specific DAMPs. Apoptosis, through phosphatidylserine externalization, mediates immunologically silent clearance, typically inhibiting inflammatory responses. Pyroptosis actively releases IL-1β via GSDMD pores, while necroptosis relies on MLKL-mediated HMGB1 release; both collectively trigger a strong inflammatory cascade. This causal association between cell death modalities and inflammation intensity is prominently manifested in pathological processes such as sepsis and autoimmune diseases, both of which are of significant concern in the perioperative setting due to their potential for exacerbation and the associated increase in morbidity. This is especially relevant given that different organs exhibit varying susceptibility to specific death pathways during surgery; for instance, the heart may be more vulnerable to ferroptotic damage during ischemia-reperfusion, while the brain's sensitivity to necroptosis could underpin postoperative cognitive decline, highlighting the need for organ-tailored protective strategies. Concurrently, bidirectional regulatory mechanisms profoundly influence cell fate. TNF-α, by concentration-dependent activation of the NF-κB or RIPK1 pathways, regulates cell survival or death decisions, respectively. IFN-γ, meanwhile, enhances apoptotic sensitivity by upregulating death receptors, forming an inflammation-death feedback loop. Metabolic reprogramming, as an emerging regulatory hub, incorporates ferroptosis and cuproptosis into an interaction network. Ferroptosis relies on lipid peroxides to activate the TLR4 pathway, while cuproptosis activates the cGAS-STING axis through mitochondrial DNA release, revealing a deep association between metabolic stress and immune response. During disease progression, NETosis-released citrullinated autoantigens drive the interferon storm in SLE, the GSDMD-IL-1β auto-amplification loop exacerbates septic multi-organ damage, and lipotoxicity-induced cell death mode switching promotes NASH fibrosis progression.

Although the interplay network of cell death and inflammation plays a role in the pathological processes of various diseases and has achieved significant success in clinical applications, this field still has many shortcomings, particularly in the con-

text of perioperative medicine, where the temporal dynamics of these pathways in response to acute insults are poorly characterized and the efficacy of targeted interventions in this unique setting remains largely unexplored. Organ-specific regulatory mechanisms remain undefined. This is particularly relevant for perioperative care, as different organs exhibit varying susceptibility to ischemia-reperfusion injury and inflammatory damage during surgery (e.g., heart, brain, kidneys), and understanding these differences is key to developing organ-protective strategies. Spatial transcriptomics reveals that high expression of TREM2 in microglia promotes apoptotic clearance, while high expression of HIF-1α in HSCs amplifies cuproptosis damage. However, the core molecular switch driving these differences remains undetermined. Secondly, the quantitative threshold for cell death mode transition lacks precise definition. Normal autophagy can protect cells, but excessive autophagy induces endoplasmic reticulum stress death via the ATF4-CHOP axis. How cells regulate protective autophagy and lethal autophagy is a field worthy of in-depth study. The critical point at which autophagy shifts from a protective mechanism to a lethal program also requires systematic study. In addition, translational medicine faces many obstacles. The most critical among these is the double-edged nature of targeted therapy. Some targeted therapeutic drugs, while killing a large number of tumor cells, release massive amounts of cellular contents, leading to tumor lysis syndrome [336]. Tumor lysis syndrome can cause metabolic disorders such as hyperuricemia, hyperkalemia, hyperphosphatemia, and hypocalcemia. These metabolic disorders can further activate inflammatory responses and damage organs such as the kidneys. This paradoxical effect demands that targeted strategies precisely balance immune homeostasis. This challenge is accentuated in the perioperative period, where the efficacy of targeted interventions is contingent not only on target engagement but also on overcoming the acute physiological perturbations of surgery and anesthesia and on the time-sensitive delivery of therapies to high-risk patients, for whom rapid, point-of-care biomarkers are urgently needed. At the same time, insufficient research into inter-organ immune crosstalk networks limits our mechanistic understanding. For example, the mechanism by which the gut microbiota metabolite butyrate inhibits intestinal epithelial necroptosis and alleviates arthritis via the GPR109A receptor has not yet been elucidated [337]. Furthermore, inefficient drug delivery severely impacts therapeutic efficacy. Systemic administration of RIPK1 inhibitors leads to immune homeostasis disruption, while the lack of liver/brain-specific delivery systems constrains precise intervention. Moreover, current research mostly focuses on single organs or specific disease models, lacking a comprehensive understanding of cell death and inflammation in complex physiological and pathological environments. How to integrate these findings into our understanding of systemic inflammatory responses, and consider the interactions between different organs, remains a challenge.

Future research needs to explore more deeply the precise molecular mechanisms of cell death and inflammation, with particular attention to the spatiotemporal dynamic characteristics of signaling pathways. For example, using high-resolution imaging techniques and biosensors, researchers can monitor the activation, localization, and interaction of key molecules during cell death in real-time, thereby revealing the intricate mechanisms that regulate inflammatory responses. This research direction focuses on the spatiotemporal dynamic characteristics of signaling pathways. Emerging technologies such as single-cell sequencing and spatial transcriptomics provide new perspectives for studying cell death and inflammation. These technologies can help researchers identify specific regulatory mechanisms in different cell subpopulations, clarify which cell types are more prone to certain types of cell death, and understand how these cells interact with other immune cells, influencing the progression of inflammatory responses. To better simulate the human physiological environment, researchers need to develop and optimize human-like three-dimensional culture systems, such as organoids and organ-on-a-chip models. These models can more realistically reflect cell-cell interactions, the influence of the ECM, as well as the gradient distribution of nutrients and oxygen, thereby enabling more accurate study of the pathophysiological mechanisms of cell death and inflammation.

Elucidating the interaction mechanisms between cell death and inflammation not only provides a unified molecular model of "death mode-receptor activation" for major diseases but also promotes targeted therapy to shift from single-pathway blockade to spatiotemporally precise intervention. This precision approach is paramount in perioperative medicine, where interventions must be acutely timed to mitigate injury during surgical stress without compromising essential healing processes and immune defense in the postoperative phase. With subcellular dynamic tracing techniques resolving millisecond-level signaling events, organ-targeted delivery systems overcoming biological barriers, and multi-omics integrated AI models reconstructing disease trajectories, the translational gap between basic discoveries and clinical practice will be bridged. The ultimate establishment of an "dynamic monitoring-targeted intervention-efficacy evaluation" individualized treatment system will open up new treatment strategies for medical challenges such as multiple organ failure and the chronic inflammation vicious cycle. Translating this systems approach into the perioperative environment, potentially through rapid point-of-care biomarkers of cell death and inflammation, could enable real-time risk stratification and guide personalized prophylactic or therapeutic strategies to protect high-risk surgical patients.

ABBREVIATIONS

4-HNE, 4-hydroxynonenal; AA, arachidonic acid; ACSL4, acyl-CoA synthetase long-chain family member 4; AIM2,

absent in melanoma 2; ALOXs, arachidonate lipoxygenases; AMPK, AMP-activated protein kinase; Apaf-1, apoptotic protease activating factor 1; APC, antigen-presenting cell; ASC, apoptosis-associated speck-like protein containing a CARD; ATG, autophagy-related; BCL-2, B-cell lymphoma 2; BCL-xL, B-cell lymphoma-extra large; cGAS, cyclic GMP-AMP synthase; CLRs, C-type lectin receptors; DAMPs, damage-associated molecular patterns; DCs, dendritic cells; DLAT, dihydrolipoyl acetyltransferase; DIM, 3,3'-diindolylmethane; ECM, extracellular matrix; ERK, extracellular signal-regulated kinase; FADD, Fas-associated death domain protein; FasL, Fas ligand; FDX1, ferredoxin 1; FLS, synovial fibroblasts; GSDMD, gasdermin D; GSDMD-N, gasdermin D N-terminal domain; GSH, glutathione; GPX4, glutathione peroxidase 4; GPR109A, G protein-coupled receptor 109A; HMGB1, High Mobility Group Box 1; HSCs, hepatic stellate cells; IBD, inflammatory bowel disease; ICD, immunogenic cell death; IRF3, interferon regulatory factor 3; LPS, lipopolysaccharide; LC3, microtubule-associated protein 1A/1B-light chain 3; MDA, malondialdehyde; MAPK, mitogen-activated protein kinase; MLKL, mixed lineage kinase domain-like protein; MOMP, mitochondrial outer membrane permeabilization; mtDNA, mitochondrial DNA; mTOR, mechanistic target of rapamycin; MyD88, myeloid differentiation primary response 88; NASH, non-alcoholic steatohepatitis; NETs, neutrophil extracellular traps; NETosis, NET formation cell death; NLRP3, NLR family pyrin domain containing 3; NLRs, NOD-like receptors; NOD2, nucleotide-binding oligomerization domain-containing protein 2; NLRP1b, NLR family pyrin domain containing 1b; NLRC4, NLR family CARD domain containing 4; PAMPs, pathogen-associated molecular patterns; PCD, programmed cell death; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PI3K, phosphatidylinositol 3-kinase; PI3P, phosphatidylinositol 3-phosphate; POCD, postoperative cognitive dysfunction; PRRs, pattern recognition receptors; RA, rheumatoid arthritis; RIPK1, receptor-interacting serine/threonine-protein kinase 1; RIPK3, receptor-interacting serine/threonine-protein kinase 3; RLRs, RIG-I-like receptors; ROS, reactive oxygen species; SLE, systemic lupus erythematosus; SPMs, specialized pro-resolving mediators; STAT, signal transducer and activator of transcription; STING, stimulator of interferon genes; TFR1, transferrin receptor 1; TGF-β, transforming growth factor beta; TLRs, Toll-like receptors; TNF, tumor necrosis factor; TNFR1, tumor necrosis factor receptor 1; TRADD, TNF receptor type 1-associated death domain protein; Tregs, regulatory T cells; TRPV4, transient receptor potential vanilloid 4; T1DM, type 1 diabetes mellitus; ZBP1, Z-DNA binding protein 1.

DECLARATIONS

Author contributions

Jiaqi Li, Chenglong Zhu, Yan Liao and Xuan Yin contributed to the manuscript writing and figure preparation; Rui Zhao and Changli Wang designed the work; Doblin Sandai, Haoling Zhang and Wangzheqi Zhang supervised the work. All authors have read and approved the final manuscript.

Funding

This research received no external funding.

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 details, images, or videos relating to an individual person.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

Not applicable.

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REVIEW ARTICLE

Fractional exhaled nitric oxide in respiratory diseases

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Received October 27, 2025; Accepted December 4, 2025; Published March 31, 2026

DOI: 10.61189/898430njufld

Abstract

Fractional exhaled nitric oxide (FeNO) is a simple, sensitive and non-invasive marker that can monitor eosinophilic airway inflammation. Its levels are influenced by height, weight, gender, ethnicity and smoking status. FeNO was first introduced as a biomarker to assist in asthma detection, and now plays an important auxiliary role in its diagnosis, management, and prognosis evaluation, including treatment guidance and drug efficacy assessment. With continuous exploration, exhaled nitric oxide has demonstrated utility in a variety of respiratory diseases, including chronic obstructive pulmonary disease, chronic cough and interstitial lung disease. It can also serve as a biomarker after lung surgery to indicate the risk of postoperative complications and impaired lung function. This article summarizes the clinical value of exhaled nitric oxide in common pulmonary diseases, aiming to provide a reference for its clinical application.

Keywords: Fractional exhaled nitric oxide, Asthma, Interstitial lung disease, Postoperative recovery, Clinical application

1 INTRODUCTION

Fractional exhaled nitric oxide (FeNO) is a non-invasive biomarker generated by the airway epithelium and pulmonary parenchyma. Its concentration closely correlates with type-2 inflammation, reflects eosinophilic airway inflammation and airway hyper-responsiveness (AHR), and plays a pivotal role in the pathophysiology of airway inflammatory diseases [1]. In recent years, FeNO has emerged as a non-invasive and highly sensitive biomarker, with its clinical utility in respiratory diseases being increasingly recognized. This review synthesizes the current evidence on the value of FeNO in common pulmonary disorders and aims to provide evidence-based recommendations for the diagnosis and management of these conditions.

2 PRINCIPLES AND METHODOLOGY OF FENO MEASUREMENT

2.1 Mechanisms of nitric oxide (NO) generation and its biological significance

FeNO measurement quantifies the NO concentration in exhaled breath, thereby providing an objective estimate of airway inflammation. It is particularly applicable to type-2 inflammation driven by T-helper-2 cells and their downstream cytokines. During eosinophilic airway inflammation, inducible nitric oxide synthase (iNOS) is markedly up-regulated, resulting in elevated FeNO levels and establishing FeNO as an internationally recognized biomarker of type-2 airway inflammation. Beyond reflecting inflammatory activity, FeNO reliably pre-

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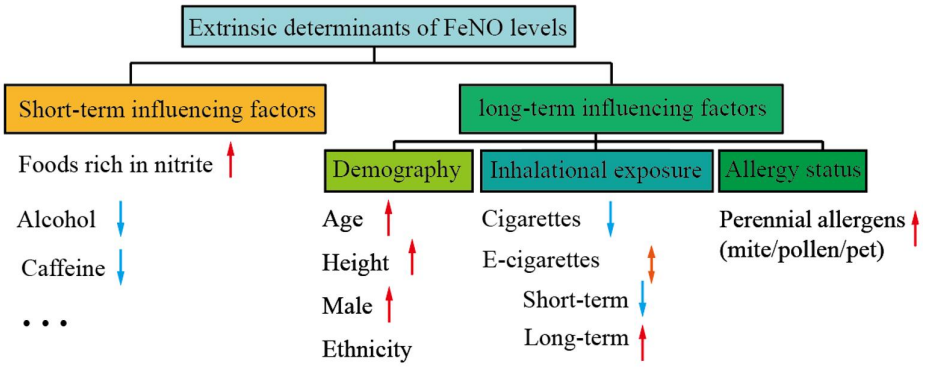

Figure 1. Determinants of FeNO. Short-term determinants: nitrite-rich foods acutely raise FeNO, whereas alcohol and caffeine transiently reduce it; long-term determinants: advancing age, greater height, male sex, chronic e-cigarette use and perennial allergen sensitization increase FeNO; conversely, cigarette smoking and short-term vaping lower FeNO. FeNO, Fractional exhaled Nitric Oxide.

dicts the therapeutic response to inhaled corticosteroids (ICS) and to biologics targeting the type-2 inflammatory pathway. Non-invasive and easy to perform, FeNO testing has become a routine tool for diagnosis, phenotyping, and management of respiratory diseases—especially asthma [2].

2.2 Determinants

Determinants of FeNO can be broadly classified into short-term and long-term influences (**Figure 1**).

• Short-term determinants

The ingestion of nitrite-rich foods, alcohol, or caffeine produces transient changes in FeNO within 3 h. Nitrite is rapidly reduced to NO by oral commensal bacteria, leading to an acute rise in exhaled NO. By contrast, both alcohol and caffeine down-regulate iNOS activity, causing a brief fall in FeNO.

• Long-term determinants

Demographic variables: FeNO increases with age and height, particularly in males, and exhibits significant inter-ethnic variation [3].

Tobacco and vaping: Cigarette smoking lowers FeNO in a dose-dependent manner [4]. The high NO concentration in smoke induces negative-feedback inhibition of iNOS, thereby reducing endogenous NO production. Consequently, the FeNO cut-offs that yield 90% specificity or 90% sensitivity are approximately 30% lower in current or former smokers than in never-smokers [5].

Biphasic effect of electronic cigarettes: Rizik et al. reported an acute fall in FeNO (median 11 parts per billion [ppb] vs 9.7 ppb, p=0.024) 30 min after a single vaping session in healthy volunteers, whereas Sompa et al. observed higher FeNO in chronic users (median 11 ppb vs 14 ppb, p=0.04), a finding consistent with airway inflammation after prolonged exposure [6, 7].

Perennial allergens: Long-term sensitisation, distinct from seasonal exposure, markedly elevates FeNO. Two large Swedish prospective cohorts—one in adults and one in adolescents—demonstrated significant increases in FeNO in individuals sensitised to animal dander, house-dust mite, or pollen, underscoring the need to consider allergen exposure when interpreting FeNO values [8, 9].

2.3 Clinical interpretation criteria for FeNO

The American Thoracic Society and the European Respiratory Society have established technical standards for FeNO measurement: The subject is required to exhale into the analyzer at a constant flow rate of 50 mL/s for at least 6 seconds if they are adolescents or adults (≥12 years), and for at least 4 seconds if they are children (<12 years). The results from repeated maneuvers are averaged and expressed in ppb. Reference ranges are 5-20 ppb for children and 5-25 ppb for adults [10].

Building on these standards, the 2017 European Respiratory Society guidelines further refined the assessment of NO from different airway compartments by specifying protocols for:

• Concentration of alveolar exhaled nitric oxide (CaNO).

• Fractional exhaled nitric oxide at an expiratory flow rate of 200 mL/s (FeNO 200).

• Fractional nasally exhaled nitric oxide (FnNO).

These additions enable a comprehensive evaluation of both central and peripheral airway inflammation.

3 TRADITIONAL APPLICATIONS OF FENO IN CHRONIC RESPIRATORY DISEASES

3.1 FeNO in bronchial asthma

3.1.1 FeNO and the pathobiology of asthma

Bronchial asthma is a chronic respiratory disease characterised by airway inflammation, AHR and remodelling. Type-2 inflammation is driven by interleukin (IL)-4, IL-5 and IL-13 released from T-helper 2 cells and group 2 innate lymphoid cells, resulting in eosinophilic infiltration, goblet-cell hyperplasia, AHR and IgE synthesis [11]. Type-2 airway inflammation is present

Table 1. Value of FeNO in asthma

Sub-topic	Key evidence	Ref
Pathobiology	FeNO produced by IL-4/13-driven iNOS-2; correlates with sputum eosinophils & AHR; usable even when FEV_1<50% pred.	[10, 14, 15]
Typical asthma diagnosis	40 ppb cut-off: pooled Se 0.81, Sp 0.83; +LR 4.8; threshold falls to 35 ppb if smokers excluded.	[21, 22]
CVA	Meta-analysis AUC 0.87; adult threshold 32 ppb (Se 0.75, Sp 0.80); combining with MMEF raises AUC to 0.90-0.92.	[24, 26, 27]
ACOS	FeNO≥25 ppb distinguishes ACOS from "pure" COPD (Se 70-74%, Sp 75-77%); OR 3.8.	[30, 31]
Exacerbation risk	Baseline ≥50 ppb→1.54-fold higher rate within 1 yr; HR rises to 3.62 if blood eos ≥150 μL^{-1} also present.	[34, 35]
Lung-function decline	Every 10 ppb increment→extra 19 mL FEV_1 loss over 2 yr; baseline FeNO explains 74% of 5-yr variance.	[37, 38]
ICS monitoring	≥20% fall after 4-w low-dose ICS predicts good control at 1 yr (OR 4.2) and unmasks non-adherence.	[33, 40]
Biologic guidance	Dupilumab: FEV_1 gain 320 mL vs 140 mL and 70% vs 35% exacerbation reduction when baseline ≥50 ppb vs <25 ppb.	[42, 43]
Limitations	Non-Type-2 asthma (30-50% adults) usually FeNO<25 ppb; overlap zone 25-40 ppb seen in 20% healthy controls.	[14, 39]

Note: CVA, Cough-Variant Asthma; ACOS, Asthma–COPD Overlap Syndrome; AHR, Airway Hyperresponsiveness; AUC, Area Under the Curve; COPD, Chronic Obstructive Pulmonary Disease; Eos, Eosinophils; FEV_1, Forced Expiratory Volume in the first second; HR, Hazard Ratio; ICS, Inhaled Corticosteroid(s); IL-4/13, Interleukin-4/Interleukin-13; iNOS-2, Inducible Nitric Oxide Synthase 2; LR, Likelihood Ratio; MMEF, Maximal Mid-Expiratory Flow; OR, Odds Ratio; Pred., Predicted (value); SE, Sensitivity; SP, Specificity.

even when symptoms are quiescent [12]. Pulmonary-function indices reflect airflow limitation but correlate weakly with the intensity of airway inflammation; consequently, a practical, non-invasive marker of active inflammation is required [13, 14].

FeNO is produced by nitric-oxide synthase (NOS)-2 induced by Type-2 cytokines. Compared with other Type-2 biomarkers (blood eosinophils, total IgE, periostin), FeNO is non-invasive, repeatable and obtainable even in patients with severe airflow obstruction [15]. FeNO correlates closely with sputum eosinophilia and methacholine AHR, and is recommended by Global Initiative for Asthma and Global Initiative for Chronic Obstructive Lung Disease to discriminate asthma from chronic obstructive pulmonary disease (COPD) [16-18]. Limitations must, however, be recognised: non-Type-2 asthma is accompanied by normal FeNO values, and overlap exists between asthmatic and healthy populations. A high FeNO increases the probability of asthma, but a low FeNO does not exclude it. The value of FeNO in asthma is summarized in **Table 1**.

3.1.2 FeNO in phenotyping and differential diagnosis

3.1.2.1 Bronchial asthma

Meta-analyses indicate moderate diagnostic accuracy for asthma [19]. In adults, an FeNO cut-off of 40 ppb yields sensitivities of 74-88% and specificities of 72-83% [20]. FeNO has been shown to correlate with exacerbation frequency and can therefore be used to supplement spirometry during initial evaluation.

3.1.2.2 Cough-variant asthma (CVA)

CVA is characterised by small-airway dysfunction without overt airflow limitation [21]. A research (nine studies, 1,046 patients) found an area under the curve (AUC) of 0.87 for FeNO [22]. Paediatric thresholds range from 28.5 ppb (sensitivity 61%, specificity 83%) to 39.8 ppb (sensitivity 77%, specificity 68%) [11]. Combining FeNO with small-airway indices (maximal mid-expiratory flow, mid-expiratory flow at 25-75% of forced vital capacity, forced expiratory flow at 25% of forced vital capacity [FEF25%], the difference between resistance at 5 Hz and 20 Hz, and reactance at 5 Hz) raises the AUC to 0.90-0.92 and significantly reduces false-negative results [23-25].

3.1.2.3 Asthma–COPD overlap syndrome (ACOS)

ACOS exhibits features of both diseases. In elderly patients with chronic airway symptoms, FeNO distinguishes ACOS from "pure" COPD [26]. Two prospective studies identified optimal thresholds of 22.5 ppb and 25.5 ppb (sensitivity 70-74%, specificity 75-77%) [27, 28]. An FeNO cut-off of 27 ppb was recorded in 83% of ACOS patients versus 52% of COPD patients and 21% of controls ($p<0.01$), without significant difference from the asthma group (88.6%) [15].

3.1.3 FeNO-guided prognosis

Longitudinal cohort studies have shown that FeNO falls progressively during the first 12 months of guideline-based asthma therapy, mirroring the waning of airway inflammation [29].

In the BUSSE cohort (n=1,026 moderate-to-severe asthma), patients with baseline FeNO≥50 ppb experienced a 1.54-fold higher exacerbation rate over the following year than those with FeNO<25 ppb; the hazard ratio was 1.33 for the intermediate group (25-50 ppb) [30]. When FeNO≥25 ppb was combined with peripheral-blood eosinophils ≥150 cells cells/μL, the exacerbation risk rose to 3.62-fold relative to patients with both values below these cut-points [31]. In head-to-head comparisons, FeNO outperformed blood eosinophils and serum periostin as a predictor of future exacerbations [32].

Elevated FeNO is also associated with accelerated forced expiratory volume in the 1st second (FEV_1) decline. In the 3751-patient PORSBJERG cohort, FeNO alone explained 74.3% of the variance in five-year FEV_1 loss; adding blood eosinophils marginally improved the model (adjusted R^2=0.751) [33]. Similarly, Nerpin et al. reported that every 10-ppb increment in FeNO was linked to an additional 19 mL decline in FEV_1 over two years [34].

Cross-sectional data, however, do not support the use of FeNO to grade current clinical severity. Naranjo-Vallejo found no difference in mean FeNO between Global Initiative for Asthma-defined mild (62.7 ppb) and moderate (54.8 ppb) asthma, and no correlation with Asthma Control Test scores [35].

Collectively, these data indicate that—while a raised FeNO forecasts future exacerbations and lung-function decline—it cannot standalone stratify current symptom severity and should be interpreted alongside other biomarkers.

3.1.4 FeNO for monitoring pharmacotherapy

ICS suppress Type-2 airway inflammation and rapidly reduce FeNO. A ≥20% fall in FeNO after 4 weeks of low-dose ICS predicts good long-term control and adherence [36]. The Scottish Consensus advocates a "FeNO-suppression test" (repeat measurement after 4 weeks ICS) to uncover non-adherence or incorrect inhaler technique [37].

Baseline FeNO is a continuous modifier of ICS responsiveness: the higher the pretreatment value, the greater the clinical and anti-inflammatory gain. A meta-analysis of six ICS trials in COPD showed a mean ~6.3 ppb reduction in FeNO overall, but a reduction of -14.6 ppb in the subgroup with baseline FeNO≥25 ppb [38].

FeNO is equally useful when biologics are introduced. In a real-world study of 30 severe asthma patients receiving tezepelumab, median FeNO fell from 35 ppb to 19 ppb by 24 weeks, concomitant with improved FEV_1 and reduced exacerbations [39]. For dupilumab, the greatest FEV_1 gain (~320 mL) and exacerbation reduction were observed when baseline FeNO exceeded 50 ppb, although benefit was already evident above 20 ppb [40].

Thus, FeNO serves as a dynamic read-out of both ICS and biologic efficacy, and can guide escalation or de-escalation decisions within a treatable-traits framework.

3.2 Inflammatory assessment in COPD

COPD is a prevalent obstructive lung disorder that affects approximately 300 million people worldwide [41]. The pathological spectrum comprises obstructive bronchiolitis, emphysema, or a combination of both, with the small airways and lung parenchyma representing major sites of inflammation [42, 43].

Early investigations suggested an association between COPD and elevated exhaled NO; however, subsequent studies have challenged this view. Lehouck et al. observed that multiple-flow exhaled NO measurements did not discriminate COPD among 151 current or former smokers, and Bazeghi et al. questioned the utility of FeNO as a marker of local COPD-related inflammation [44-46]. A plausible explanation is that early-stage COPD generates a localized inflammatory milieu that fails to up-regulate iNOS, thereby leaving FeNO unchanged.

Nevertheless, a high FeNO value constitutes an independent risk factor for incident COPD [47]. During acute exacerbations, intensified and disseminated airway inflammation can raise FeNO, providing a window on disease severity. In a two-year longitudinal cohort, Högman et al. demonstrated that persistently abnormal FeNO was associated with significantly lower lung function and more extensive airway obstruction/destruction [48]. Similarly, Romero-Linares reported that patients with elevated FeNO exhibited markedly reduced survival and a three-fold higher risk of moderate-to-severe exacerbations (HR 3.01, 95% CI 1.83-4.93; p<0.001) [49].

Collectively, these data indicate that while FeNO is not elevated in all COPD patients, chronically increased FeNO identifies a distinct inflammatory phenotype characterized by accelerated lung-function decline and adverse clinical outcomes. This discrepancy may stem from the heterogeneous inflammatory profiles observed in COPD, which encompass macrophagic, neutrophilic, eosinophilic, T-cell- and B-cell-driven patterns in varying combinations. As reported by David et al., only 20-40% of patients exhibit a type-2 inflammatory endotype characterized by elevated airway eosinophils [50]. The precise mechanisms underlying type-2 inflammation in COPD remain elusive; nevertheless, this endotype is more prevalent during acute exacerbations [51]. Consequently, elevated FeNO appears to be a discriminative biomarker specifically in acute exacerbations of COPD (AECOPD) rather than in stable disease, underscoring its potential as a prognostic biomarker in COPD management (**Figure 2**).

3.3 Application of FeNO in the differential diagnosis of chronic cough

Chronic cough is defined as cough lasting more than 8 weeks in adults. The most common aetiologies in adults are upper-air-

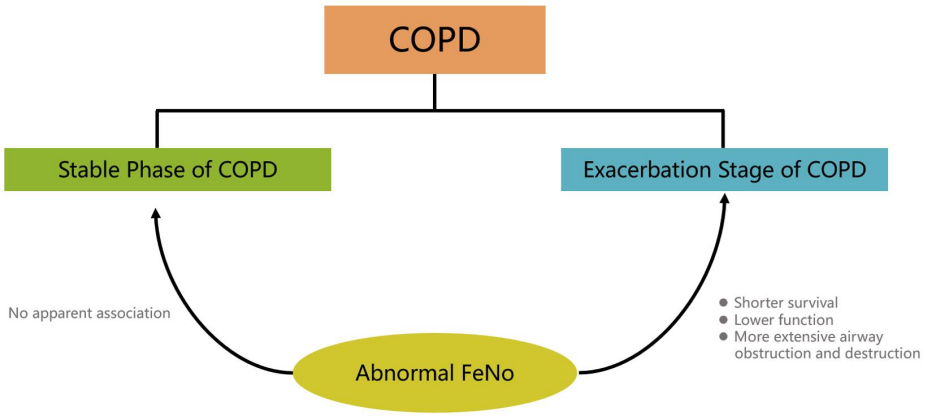

Figure 2. Utility of FeNO in COPD. Abnormal FeNO exerts no significant influence on patients with stable COPD; however, during acute exacerbations, it is associated with shortened survival, lower lung function, and more extensive airway obstruction and destruction. FeNO, Fractional exhaled Nitric Oxide; COPD, Chronic Obstructive Pulmonary Disease.

way cough syndrome, CVA, eosinophilic bronchitis (EB), and gastro-oesophageal reflux-related cough (GERC) [52]. CVA has been detailed in Section 3.1.3 and is not repeated here.

Experimental data indicate that eosinophil-derived airway hyper-innervation, stimulation of vagal C-fibres, and the interaction between major basic protein and the TRPV1 receptor may contribute to chronic cough. FeNO, reflecting airway eosinophilic inflammation, has therefore been investigated as a non-invasive tool for identifying the underlying cause and predicting cough severity [53]. Beck et al. reported a positive correlation between FeNO and mucus-plug scores [54].

EB is characterised by irritative dry cough or scanty sputum, with a sputum eosinophil count ≥2.5%. Ninety percent of EB patients exhibit elevated FeNO, and serial FeNO measurements track airway inflammation and forecast responsiveness to ICS [55].

GERC is another major cause of chronic cough, presumably via reflux-mediated activation of the oesophago-bronchial vagal pathway or micro-aspiration into the upper airways. Recent work showed that proximal-airway nitric oxide (CaNO) is significantly higher in GERC patients; higher CaNO values were associated with more frequent proximal reflux episodes and elevated pepsin levels in induced-sputum supernatants. Furthermore, CaNO exhibited moderate predictive value for the efficacy of intensive anti-reflux therapy; after a course of treatment, CaNO declined in parallel with cough resolution, suggesting that CaNO may serve as a non-invasive biomarker of peripheral-airway inflammation in GERC [56].

In summary, FeNO has demonstrated modest utility in the diagnosis and prognosis of selected chronic-cough phenotypes; however, large-scale studies investigating its pathogenic mechanisms and discriminative value across the major chronic-cough subtypes are still lacking and should be the focus of future research.

3.4 FeNO in interstitial lung diseases (ILDs)

3.4.1 CaNO overview

CaNO, a sub-fraction of exhaled NO obtained at low expiratory flow rates, is a simple and non-invasive index of small-airway dysfunction. At a cut-off of 5.3 ppb, CaNO predicts small-airway impairment with 72% sensitivity and 92% specificity [57]. ILDs comprise a heterogeneous group of diffuse parenchymal disorders associated with high morbidity and mortality [58]. The current classification distinguishes major, rare and unclassifiable entities, each encompassing numerous disorders. To date, most NO-ILD research has focused on two prototypic disorders: systemic-sclerosis-related ILD (SSc-ILD) and idiopathic pulmonary fibrosis (IPF). The following sections summarize the evidence for CaNO in these conditions.

3.4.2 SSc-ILD

Systemic sclerosis (SSc) is a heterogeneous connective-tissue disease in which ILD represents the leading cause of death [59, 60]. Early studies documented abnormally elevated CaNO in patients with SSc-ILD [61]. Building on this observation, Tiev et al. proposed CaNO as a diagnostic biomarker, reporting a threshold of 4.3 ppb (sensitivity 87%, specificity 59%) for detecting SSc-ILD [62].

Subsequent work has explored CaNO for monitoring disease trajectory. CaNO accurately identifies patients at risk of clinical deterioration; levels correlate directly with ILD extent and inversely with diffusing capacity for carbon monoxide, although associations with overall lung function remain inconsistent [61, 63]. According to the 2020 European consensus on SSc-ILD, high-resolution computed tomography (HRCT) is the reference standard [60]. Hoang-Duc demonstrated that changes in HRCT scores (ΔWarrick and ΔGoldin) correlated strongly with ΔCaNO ($R=0.783$, $p<0.01$), and combining CaNO with HRCT improved diagnostic accuracy [64]. Collectively, elevated CaNO reflects greater pulmonary involvement and may track SSc-ILD progression.

Notably, Wuttge observed no difference in CaNO between early-stage SSc patients with and without radiological ILD, suggesting that CaNO elevation may precede imaging abnormalities and serve as an early marker of pulmonary involvement

[65]. Murine data from Hua-Huy support this concept, showing that pulmonary inflammation occurs in early SSc and that early treatment attenuates disease development [66].

In summary, CaNO exhibits excellent sensitivity in patients with SSc-ILD, making it a valuable tool for initial disease screening; however, its suboptimal specificity necessitates combination with other diagnostic modalities to improve diagnostic accuracy. A current limitation is the absence of head-to-head comparisons between CaNO and other prognostic biomarkers in SSc-ILD [67].

3.4.3 IPF

IPF is a chronic, progressive fibrosing interstitial pneumonia that culminates in respiratory failure within 2-5 years of diagnosis [67, 68]. Periostin has emerged as a promising biomarker of disease activity and a potential therapeutic target, performing comparably to established markers KL-6 and SP-D [69].

Initial studies indicated that $FeNO_{50}$ is not useful for IPF diagnosis, whereas CaNO showed greater potential. A systematic review pooling three studies demonstrated significantly higher CaNO in IPF patients (8.5±5.5 ppb) than in controls (4.4±2.2 ppb) [70]. Cameli et al. further performed survival and progression analyses, revealing that CaNO≥9 ppb was associated with earlier mortality and disease progression; thresholds of 6 ppb and 9 ppb independently predicted death and progression, respectively [68]. In a subsequent study, the same group reported a significant correlation between CaNO and serum periostin levels, suggesting convergent biological pathways linking alveolar inflammation to fibroproliferation in IPF [71].

Although encouraging findings have emerged for CaNO in IPF, the current evidence is constrained by small, single-center retrospective studies that lack external validation cohorts. To date, only an observational association between CaNO and periostin has been demonstrated; causal mechanisms and longitudinal comparative data remain unavailable. Future investigations should delineate the specific signaling pathways linking CaNO to periostin and conduct prospective head-to-head longitudinal analyses that concurrently evaluate CaNO, periostin, KL-6, and SP-D.

4 ADDITIONAL CLINICAL APPLICATIONS OF FENO TESTING

4.1 Peri-operative risk stratification and pulmonary rehabilitation

4.1.1 Peri-operative value of FeNO in pulmonary resection

Pulmonary resection, the most commonly employed thoracic surgical technique, entails the excision of diseased lung tissue to achieve therapeutic goals [72]. However, the consequent reduction in lung volume frequently disturbs respiratory physiology; conventional thoracotomy, for example, may diminish post-operative pulmonary function by 10-40% [73]. Inflammatory cascades are triggered through multiple mechanisms, including pre-existing comorbidities and intra-operative mechanical stress (compression, retraction, and transection), which release pro-inflammatory mediators such as IL-6 and IL-8 [74, 75]. These cascades, in which type-2 inflammation is prominent, contribute to acute lung injury, impede functional recovery, and may culminate in systemic inflammatory response syndrome and post-operative pulmonary complications (PPCs) such as pneumonia and atelectasis [76].

Pre-operative FeNO can be used as an inflammatory biomarker to inform surgical planning. The Japanese Respiratory Society advocates that screening FeNO identifies covert airway inflammation and thereby facilitates optimisation of pre-operative preparation and choice of procedure [77]. Elevated pre-operative FeNO may impair early functional recovery after lobectomy. Okamoto et al. reported that lung-cancer patients with pre-operative FeNO>25 ppb exhibited lower FEV_1 and VC than controls, indicating that abnormal FeNO is associated with both obstructive and restrictive defects; spirometric indices remained inferior at 1 and 3 months post-surgery, suggesting delayed recovery [78]. By 6 months, however, the two groups converged, presumably because recovery in the normal-FeNO cohort had plateaued after the third month [79].

Abnormal FeNO also predicts PPCs with high specificity. He et al. identified pre-operative FeNO as an independent predictor of PPCs under general anaesthesia (AUC 0.835, sensitivity 62.5%, specificity 92.3%) [80]. Post-operative cough—defined as persistent cough for ~3 months and occurring in 50-94.7% of thoracic surgical patients—delays rehabilitation [81]. In Lin's prospective study of 128 non-small-cell lung-cancer patients undergoing pulmonary resection, pre-operative FeNO independently predicted post-operative cough (OR 1.106, 95% CI 1.076-1.137, $p<0.001$); participants with low FeNO reported significantly higher LCQ-MC scores at 1 month and achieved faster recovery in physical (28 vs 91 days), psychological (28 vs 60 days), social (28 vs 80 days), and total (28 vs 91 days) domains ($p<0.05$), implying that lower airway inflammation facilitates resolution of surgery-related airway irritation [82].

Collectively, pre-operative FeNO assists surgical decision-making—although it should not be the sole determinant—and predicts both functional recovery and post-operative morbidity. Nevertheless, current evidence is limited: apart from Lin's cohort, most studies address composite PPC endpoints rather than the pathobiology of individual complications, and the mechanisms through which pre-operative FeNO forecasts specific PPCs remain unexplored. Future work should elucidate these mechanisms to refine clinical practice. Moreover, although serial post-operative FeNO monitoring could provide valuable guidance for peri-operative management, relevant

evidence remains scarce. This gap largely reflects poor patient compliance with follow-up during the post-surgical period and the prevailing reliance on conventional spirometric indices for longitudinal assessment of pulmonary function [73, 83]. Consequently, studies specifically evaluating prolonged FeNO surveillance after lung resection are limited. Future investigations should therefore clarify the relationships between postoperative FeNO trajectories, pharmacological requirements, functional recovery, and the development of specific complications, so as to consolidate the clinical utility of this biomarker.

4.1.2 FeNO in lung transplantation

Lung transplantation is the definitive therapy for end-stage lung disease [84]. Mechanical trauma during procurement and implantation, together with allo-immune responses, injures airway epithelium and incites inflammation [85]. Recipients are at risk of early primary graft dysfunction and chronic complications, including allograft rejection and bronchiolitis obliterans syndrome (BOS) [86]. BOS—a fibro-proliferative obliteration of small airways mediated by immune injury, inflammatory-cell infiltration, excessive extracellular-matrix deposition, and aberrant epithelial repair—is the leading cause of late morbidity and mortality; its incidence correlates with primary graft dysfunction severity [87].

Short-term elevation of pro-inflammatory cytokines such as IL-6 predicts BOS, rejection, and reduced survival, prompting interest in early inflammatory biomarkers for risk stratification and pre-emptive therapy [88].

FeNO, a non-invasive marker of airway inflammation, may facilitate detection of acute complications after transplantation. Gashouta et al. reported an AUC of 0.93 ($p<0.001$) for postoperative FeNO>10 ppb in predicting early graft complications (sensitivity 82%, specificity 100%, positive predictive value 100%, negative predictive value 97.5%), underscoring its diagnostic utility for infection-related airway inflammation [89].

Most transplant studies have focused on FeNO for BOS prediction. Pulmonary infection and lymphocytic bronchiolitis are pivotal antecedents. Vos et al. observed elevated FeNO in patients with lymphocytic bronchiolitis, which normalised after azithromycin therapy [90].

FeNO exhibits excellent negative but modest positive predictive value for BOS. Neurohr et al., in a cohort of 116 patients with BOS, reported higher FeNO in unstable than in stable BOS (32.5±1.3 ppb vs 15.3±0.8 ppb); the threshold >20 ppb yielded positive and negative predictive values of 69.0% and 96.9%, respectively, supporting its utility for longitudinal risk stratification [91]. Cameli et al. proposed CaNO as a potential BOS biomarker; however, this finding is based on a small sample (27 stable transplant recipients) and awaits independent validation [92].

In summary, FeNO may facilitate early detection of pulmonary infection and lymphocytic bronchiolitis, thereby enabling pre-emptive intervention against BOS, and offers excellent negative predictive value for established BOS. Nevertheless, its modest sensitivity and positive predictive value necessitate integration with additional biomarkers, and larger, long-term prospective studies are required to fully delineate FeNO's role in the longitudinal management of lung-transplant recipients.

4.2 Predicting post-coronavirus disease 2019 (COVID-19) sequelae

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a highly transmissible and pathogenic coronavirus that emerged at the end of 2019, has caused a global pandemic of acute respiratory disease termed COVID-19, posing a major threat to public health [93]. A substantial proportion of survivors continue to experience chronic respiratory symptoms and persistent airway inflammation, and individuals with comorbidities such as COPD or bronchial asthma are more prone to severe COVID-19 manifestations [94, 95]. Kerget et al. demonstrated that FeNO levels were significantly higher in SARS-CoV-2-infected patients than in healthy controls ($p<0.001$) and showed a strong association with macrophage activation syndrome. Their findings indicate that SARS-CoV-2 infection up-regulates airway NOS, a process closely linked to type-2 inflammation [96].

In a subsequent study, Lior et al. stratified SARS-CoV-2-infected patients into mild-to-moderate, severe, and critical subgroups and evaluated the utility of FeNO alongside standard spirometric indices—FVC%, FEV1%, and FEF25–75%—for evaluating disease severity. FeNO and the small-airway parameter FEF25–75% were significantly lower in the severe group compared with the mild-to-moderate cohort, whereas FEV1% did not decline significantly until the critical stage. These findings indicate that FeNO, in conjunction with FEF25–75%, may serve as an early-to-mid-phase biomarker of SARS-CoV-2 progression [97]. Moreover, FeNO has the potential to predict post-recovery complications in patients with COVID-19, in whom pulmonary fibrosis represents the dominant long-term sequela of severe SARS-CoV-2 pneumonia. In a study by Ferrer-Pargada et al. that enrolled 335 patients hospitalized for severe COVID-19 pneumonia, individuals who developed fibrotic interstitial sequelae exhibited higher FeNO levels (mean 24.3 ppb versus 19.8 ppb, $p=0.002$). They established a prediction model incorporating six variables—including FeNO—which demonstrated good discriminative performance for fibrotic interstitial sequelae (AUC 0.81 for continuous FeNO, AUC 0.82 for dichotomized FeNO), highlighting the utility of FeNO in forecasting post-COVID-19 lung fibrosis [98].

4.3 Clinical application in pulmonary hypertension (PH)

PH is characterized by pathologically elevated pulmonary vascular pressure, accompanied by obstructive, sclerotic, and

vasoconstrictive remodeling of the pulmonary vasculature; dyspnea is the predominant symptom [99]. Accumulating evidence indicates that inflammation underlies all forms of PH [100].

FeNO may aid in diagnosing PH when combined with other clinical entities. Xu et al. evaluated FeNO levels in 54 patients with idiopathic pulmonary arterial hypertension (IPAH) and 78 with mixed connective-tissue-disease-associated PH; FeNO was significantly higher in the IPAH cohort. Within the IPAH group, FeNO values were lower in patients with severe disease than in those with mild PH (p=0.024) [101]. Guo and colleagues assessed FeNO for detecting PH during AECOPD; FeNO was significantly lower in AECOPD patients with concomitant PH than in those without (p=0.022) [102]. Taken together, these data suggest that FeNO may serve as a non-invasive inflammatory biomarker to facilitate phenotyping and risk stratification of PH across diverse clinical contexts.

5 SUMMARY

Exhaled NO, synthesized by NOS, offers a rapid and non-invasive window onto airway inflammation. In traditional respiratory medicine, FeNO was first exploited to identify type-2 asthma and is now validated for phenotyping asthma subtypes, stratifying exacerbation risk, forecasting lung-function decline, guiding ICS dosing, and monitoring biologic therapy. Beyond its use in asthma, FeNO has emerged as a useful adjunct in COPD, chronic cough, and ILD. While it cannot distinguish COPD from other entities, it predicts severe outcomes of AECOPD; in chronic cough, preliminary data—while scarce and fragmented—suggest diagnostic and prognostic value for selected phenotypes; and CaNO, reflecting alveolar NO flux, can differentiate SSc-ILD and IPF from healthy controls and correlates with disease progression.

Novel applications are now being explored. During the perioperative phase of lung resection, FeNO serves as an inflammatory biomarker to help select the surgical approach and to predict post-operative FEV_1 decline and complications. After lung transplantation, FeNO exhibits excellent negative-predictive value for BOS and, when combined with other biomarkers, may reduce mortality. In SARS-CoV-2 infection, FeNO assists in identifying severe disease and in forecasting selected long-term sequelae. Emerging data also hint at diagnostic utility in PH.

As a biomarker of type-2 inflammation, FeNO is sensitive to this pathway, non-invasive, rapid, and office-friendly. However, important limitations remain. Reliable measurement demands adequate patient cooperation; individuals with cognitive or severe physical impairment often cannot perform the the required maneuver. Current analysers are bulky and non-portable, excluding bed-bound or ventilated patients. Future development should therefore focus on simplified, miniaturised devices. Moreover, NO reflects only type-2 inflammation; expanding the exhaled panel to include CO, H_2S, and other volatile species could broaden the clinical spectrum of breath analysis.

Overall, exhaled NO analysis is a convenient, reproducible, and sensitive tool whose indications now extend from outpatient asthma care to a wide array of respiratory diseases and peri-operative settings, and continue to grow.

DECLARATIONS

Author contributions

Chong Zhou: conceptualization, literature search, manuscript drafting and revision. Xiao Qi Li: data curation, figure preparation, and critical review. Jinjing Xia: supervision, project administration, and final approval of the manuscript. Menglan Hao: Provided valuable suggestions on the structure and content of the article. Shuqing Chang: Provided valuable suggestions on the structure and content of the article. All authors read and approved the final version.

Funding

The authors declare that they have no funding Information.

Data availability

Not applicable.

Ethics approval and consent to participate

Not applicable.

Consent for publication

On behalf of all co-authors, I confirm that this manuscript has not been published elsewhere (except in the form of an abstract, a published lecture, or an academic thesis) and is not currently under consideration for publication in any other journal. All authors have read and approved the final version of the manuscript and agree to its submission to PPM.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

We thank all individuals who contributed to this work: the study participants for their generosity, our colleagues for their insightful suggestions, and the laboratory and administrative staff for their steadfast support throughout every stage of the project.

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Perioperative Precision Medicine

Zentime

PERSPECTIVE

Modern trends in perinatal and obstetric anesthesia: The dual challenges of risk management and resource optimization

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Received December 10, 2025; Accepted February 28, 2026; Published March 31, 2026

DOI: 10.61189/056184yqnaiu

Abstract

Obstetric and perinatal anesthesia, while crucial for maternal and neonatal health, carries inherent risks that necessitate careful management and resource optimization to ensure patient safety. Improvements in anesthesia methods, ultrasound-assisted blocks, multimodal analgesia and accelerated rehabilitation protocols have enabled individualized and precise control of postoperative pain to be achieved more readily. Risk analysis, dynamic observation, and personalized intervention can all significantly lower the rates of hypotension, nausea and vomiting, and nerve injury. International practice variations highlight how resource availability—including staffing levels and expertise—and adherence to clinical guidelines profoundly impact maternal and neonatal outcomes, underscoring that scientific resource optimization is essential for ensuring safety and enhancing efficiency. This perspective discusses the current trends in perinatal anesthesia, focusing on risk minimization and resource utilization. By synthesizing recent advancements and practice variations, we aim to offer a conceptual framework and valuable insights for clinicians making management decisions.

Keywords: Perinatal anesthesia, Regional analgesia, Risk management, Resource optimization, Maternal and neonatal safety, Multimodal analgesia

1 INTRODUCTION

Perinatal and obstetric anesthesia is an important part of contemporary medicine that directly affects the health of mother and neonates as well as perioperative management. While often used interchangeably with obstetric anesthesia, perinatal anesthesia specifically emphasizes the continuous care spectrum affecting both the mother and the neonate immediately before, during, and after delivery, highlighting the unique physiological and safety considerations for this dyad. Over the past several decades, anesthetic practice has evolved from being experience-based to a scientific, evidence-supported, personalized, and multidisciplinary model. All techniques, including ultrasound-guided anesthesia and epidural anesthesia, are now performed by experienced clinicians, with efficacy evaluated using key performance indicators such as first-attempt success rates, the number of puncture attempts, procedure duration, and patient satisfaction scores. Simultaneously, safety is assessed by monitoring adverse events like vascular puncture or sensory deficits [1, 2].

In recent years, individualized postoperative pain management has become a reality through the optimization of low-dose medications, selective use of regional anesthesia, ultrasound-guided nerve blocks, multimodal analgesia approaches, and the integration of patient-controlled analgesic devices into

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enhanced recovery protocols. However, while recent reviews have expertly detailed advances in neuraxial techniques or Enhanced Recovery After Surgery protocols, the unique focus of this perspective is to synthesize the symbiotic relationship between risk management and resource optimization, arguing that the latter is the foundational enabler of the former. We aim to offer a conceptual framework and valuable insights for clinicians making management decisions.

2 CURRENT STATUS AND DEVELOPMENT TRENDS IN PERINATAL ANESTHESIA

2.1 Innovations and applications in anesthesia technology

Advances in technology and pharmacology have been pivotal in improving maternal and neonatal safety and in providing pain relief. For instance, adjunctive measures like preoperative oral carbohydrates have been shown to significantly reduce patients' thirst, hunger, and anxiety, contributing to overall comfort and facilitating postoperative recovery [3]. The precise application of regional anesthesia techniques (epidural, spinal, and combined spinal-epidural) and the development of low-dose drug protocols have helped to minimize the potential side effects associated with general anesthesia. The integration of ultrasound in the implementation of nerve blocks has demonstrably increased both procedural safety and success rates.

Furthermore, the emergence of multimodal analgesia regimens, patient-controlled analgesia (PCA), and enhanced recovery programs has allowed for the better individualization of postoperative pain management (Supplementary Table 1). The table presents an overview of the most commonly used anesthesia techniques as well as special considerations dependent on anesthesia type pertaining to the perioperative period, such as regional anesthesia, low-dose drug regimens, and applications of ultrasound-guided nerve blocks. This forms a solid basis for updating perinatal anesthesia practice. Lessons from the SARS-CoV-2 pandemic have also underscored the value of neuraxial anesthesia in minimizing aerosol-generating procedures, highlighting its critical role in pandemic preparedness protocols for future public health crises [4].

2.2 Global practice variations

Significant global disparities exist in perinatal anesthesia practice, driven by differences in resource availability, staffing levels and expertise, clinical guidelines, and institutional culture. In high-income settings, interventions such as low-dose spinal anesthesia combined with epidurals and PCA pumps are common, intended to maximize maternal hemodynamic stability and minimize adverse neonatal events. Conversely, resource-limited settings are often constrained by a lack of equipment, drugs, and trained personnel. In some regions, a high cesarean section rate is ascribed not only to clinical indications but also to sociocultural factors, such as fear of labor, in conjunction with a shortage of anesthesiologists that makes epidural anesthesia a less available option [5]. As demonstrated by quality improvement projects, standardization of care and targeted interventions can lead to a consistent decrease in maternal and neonatal mortality, underscoring the impact of systematic approaches [6]. Acknowledging these global differences is essential for developing tailored risk management strategies and optimizing resource allocation to promote more balanced and equitable development in perinatal anesthesia worldwide.

3 APPLICATION OF RISK MANAGEMENT IN PARINATAL ANESTHESIA

3.1 Risk assessment tools and methods

Systematic risk assessment is essential to guarantee maternal and neonatal safety. An effective risk management workflow begins with a proactive preoperative assessment, using systematic tools to stratify patient risk. For instance, validated scoring systems can identify parturients at high risk for complications like hypotension or nausea. Advanced methods, such as machine-learning models (e.g., MLPIAAR), can further refine these predictions, guiding personalized drug and dosage selection even before the procedure begins. Mathematical models have also been employed to define optimal pump settings for programmed intermittent epidural bolus to enhance efficacy and safety [7]. This initial step sets the foundation for a tailored anesthetic plan. Evidence also guides the selection of adjuncts; for example, dexamethasone and flurbiprofen axetil can decrease the incidence of postoperative vomiting, whereas hydromorphone PCA may increase it [8]. The integration of electronic medical records and artificial intelligence-based dynamic monitoring further enables these strategies to be optimized in real-time.

3.2 Preventive measures and response strategies

Risk management extends into the operating room with a system of "triggers and responses" based on continuous monitoring. Predefined changes in maternal hemodynamics, fetal heart rate, or anesthetic depth (the trigger) should automatically initiate a protocolized response, such as a fluid bolus or vasopressor administration. This proactive approach ensures timely intervention based on objective quality metrics rather than delayed clinical signs. A primary risk during neuraxial anesthesia is maternal hypotension, which can impair uteroplacental perfusion. Pharmacological prevention is therefore a key component of risk management. Evidence from clinical studies suggests that a prophylactic infusion of methoxamine (2.00 μg/kg/min) is as effective as norepinephrine (0.10 μg/kg/min) in maintaining maternal hemodynamics without adversely affecting neonatal outcomes in low-risk parturients [9]. In contrast, other studies have found that certain interventions, like two doses of ondansetron, apparently did not prevent hypotension during cesarean section under ropivacaine epidural anesthesia [10].

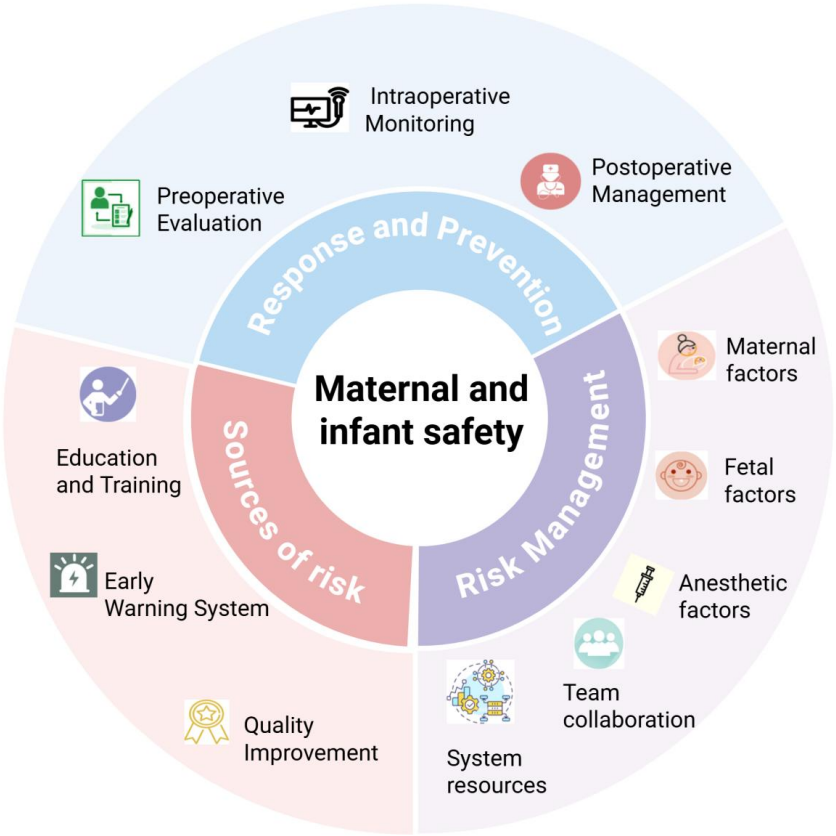

Figure 1. Perioperative risk management framework. This figure provides a structured overview of the fundamental components of perioperative risk management. It is composed of three key clinical process phases—preoperative planning, intraoperative execution and postoperative management—and incorporates comprehensive supports including education and training, an early warning system and team cooperation. The framework is designed to minimize maternal and neonatal harm by increasing quality of care through recognition of internal and external risk factors (e.g., anesthesia-specific, systems-based).

Meanwhile, perineal ultrasound measurements have revealed that epidural analgesia has no significant impact on early postpartum pelvic floor function, providing an objective tool for evaluating labor progress and guiding anesthetic application safely [11].

3.3 Recent advances in postoperative complication management

The treatment of postoperative complications has moved from being purely experience-based to an evidence-based, patient-specific standard of care. The early recognition of high-risk factors, introduction of protocolized care pathways, administration of multimodal analgesia, and comprehensive monitoring can significantly lower the occurrence of hypotension, nausea, vomiting, and neurological damage. This evidence-based approach also informs de-implementation of ineffective practices. For example, studies have demonstrated that higher doses of antibiotics do not significantly reduce cesarean section surgical site infection rates, nor does skin preparation with azithromycin reduce them [12, 13]. This paper introduces a conceptual framework for perioperative risk management that spans the entire process, from preoperative assessment to postoperative care (**Figure 1**). This framework integrates risk prediction models, individualized drug regulation, early warning systems, and robust education and training to systemically promote safety for both mothers and neonates.

4 RESOURCE ALLOCATION AND OPTIMIZATION STRATEGIES

4.1 Rational allocation of human resources

The logical and rational deployment of human resources is critical to maintaining maternal and neonatal safety with high efficiency. Anesthesiologists, midwives, and nurses should be assigned according to the type of surgery, level of anesthesia complexity, and patient risk profile. Creating flexible teams through layered management systems and rotation schemes ensures both routine and emergency support. For example, professional societies like the American Society of Anesthesiologists recommend staffing models that ensure the immediate availability of a dedicated anesthesia provider for every labor and delivery unit. The critical role of providers like certified registered nurse anesthetists in underserved areas exemplifies how professional autonomy can improve access to obstetric services where provider shortages exist [14]. Furthermore, regular interdisciplinary simulation training focusing on high-stakes scenarios (e.g., maternal hemorrhage, neonatal resuscitation) is essential for improving team coordination and readiness.

4.2 Integration of equipment and technological resources

Optimizing the use of equipment is crucial. For example, preoperative handheld ultrasound application can remarkably improve the first-attempt success rate of labor Combined Spinal-Epidural analgesia, reducing procedure time and improving patient experience. The integration of high-precision monitoring devices, PCA pumps, and information platforms that leverage real-time data analytics facilitates dynamic monitoring and evidence-based clinical decision support for hemodynamics, oxygenation, and anesthetic effect. This leads to an optimal operational flow and boosts the accuracy and traceability of anesthesia management [15].

4.3 Economic benefits and cost control analysis

Economic considerations and cost containment are central to resource management. The smart allocation of anesthesia equipment, drugs, and manpower—combined with clinical strategies like low-dose spinal anesthesia, multimodal analgesia, and PCA pumps—can systematically reduce drug waste, shorten hospital stays, and lower overall costs. This achieves dual clinical and economic benefits. Further efficiencies can be gained by optimizing operating room workflows, for example, ensuring the timely arrival of patients and surgical teams for preoperative assessment and communication has been shown to maximize efficiency. Scientific configuration and rationalization of all resources ensure that economic benefits and clinical quality are both promoted, supporting the sustainable development of perinatal anesthesia.

5 DISCUSSION

Advancements in perinatal and obstetric anesthesia have led to significant improvements in maternal safety, neonatal outcomes, and analgesic efficacy, driven by the development of new skills and the promotion of modern perioperative management. This work has critically evaluated technological applications, maternal and fetal outcomes, and resource utilization through the lens of risk quantification. We propose an innovative paradigm that views risk management and resource optimization as inextricably linked. The safe and effective implementation of modern anesthetic techniques, from ultrasound guidance to multimodal analgesia, depends entirely on a robust risk management framework. This framework, in turn, is fundamentally supported—or constrained—by the strategic allocation of human, technological, and economic resources. This synergy is the key to advancing perinatal care, enhancing efficiency, and ensuring the highest standards of safety for both mother and neonate.

ABBREVIATIONS

MLPIAAR, Machine-learning model for postoperative analgesia and adverse reactions; PCA, Patient-Controlled Analgesia; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2.

DECLARATIONS

Author contributions

Dezhi Guo and Jingjing Lu contributed to the manuscript writing and figure preparation. Limin Wei and Wenyi Wang supervised the work. All authors have read and approved the final manuscript.

Funding

This research received no external funding.

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 with human participants or animals performed by any of the authors.

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 competing interests.

Acknowledgements

Not applicable.

Supplementary Information

The online version (of this article) contains supplementary material available at https://doi.org/10.61189/056184yqnaiu.

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Supplementary Information

Supplementary Table 1. Overview of perinatal anesthesia techniques and their clinical applications

Anesthesia techniques	Areas of application	Clinical advantages	Potential risks/limitations	Development trends/ innovative directions	References
Intrathecal Morphine (ITM) analgesia	Postoperative analgesia following cesarean section	Improves obstetric recovery, reduces opioid consumption	Opioid-related side effects, requires monitoring	Optimizing maternal recovery in multimodal analgesia strategies	[1]
Combined Spinal-Epidural Anesthesia (CSEA) vs Continuous Epidural Anesthesia (CEA) (Ropivacaine+Sufentanil)	Labor analgesia	Rapid onset, significant pain relief, high satisfaction	Higher adverse reactions with CEA, requires skilled operation CEA	Clinical promotion and optimization of application	[2]
CSEA	Labor analgesia for natural childbirth	Reduces anxiety, significant pain relief, low stress response	Low risk of motor blockade, fewer complications	Improving pain relief safety and optimizing application	[3]
CSEA+acupoint injection	Labor analgesia	Significant pain relief, reduced medication usage	Hypotension, nausea, vomiting, and other adverse events require monitoring	Combination technique to optimize maternal-fetal inflammatory response	[4]
Dural Puncture Epidural (DPE) anesthesia	Cesarean section analgesia	Cesarean section analgesia	Rapid onset, excellent blockade, high satisfaction	Risk of hypotension and intraoperative pain requires monitoring	[5]
CSEA	Labor analgesia	Effective pain relief, controlled induction time	Increased incidence of numbness in the lateral position, increased demand for ephedrine	Optimize positioning to reduce complications	[6]
CSE anesthesia	Labor analgesia	Rapid onset, high safety	Slight increase in maternal uterine artery resistance, fetal heart rate monitoring required	Safe analgesia strategy with low-dose local anesthetics and precise monitoring	[7]
Combined subarachnoid-epidural anesthesia	Labor analgesia	Rapid onset of pain relief, high safety	Increases the risk of maternal fever during labor	Develop predictive models for early identification of high-risk parturients	[8]
Patient-Controlled Epidural Analgesia (PCEA) (Ropivacaine)	Labor analgesia	Effective pain relief, high satisfaction	Frequent pain breakthrough with low concentration	Optimize concentration and dosage regimen	[9]
PIEB+PCEA	Labor analgesia	Reduced intervention, high satisfaction	No significant increase in adverse events	Reduce medical interventions, optimize drug administration	[10]
PIEB epidural analgesia	Labor analgesia	Significant pain relief, low breakthrough pain	Prolonged second stage of labor	Optimize bolus volume and individualized dosing	[11]
DPE epidural anesthesia	Labor analgesia	Good pain relief for breakthrough pain	Experience-dependent, high technical requirements	Enhance analgesia precision and effectiveness	[12]
PIEB+PCEA	Labor analgesia	Reduced drug dosage	Increased staff workload	Optimize the balance between analgesia and workload	[13]
CSE (low-dose ropivacaine - sufentanil)	Labor analgesia	Rapid onset, significant pain relief, low demand for supplemental analgesia	Spinal opioid dose-related adverse reactions	Optimize analgesia, reduce breakthrough pain, multi-center validation	[14]
ClearSight guided Goal-Directed Fluid Therapy (GDFT)	Spinal anesthesia for cesarean section	Reduces nausea	Does not improve hypotension	Optimize fluid management strategies	[15]

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PERSPECTIVE

Rapid anesthesia assessment and clinical emergency management in emergency surgery

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Received December 21, 2025; Accepted March 13, 2026; Published March 31, 2026

DOI: 10.61189/891717grkkpo

Abstract

Emergency surgery anesthesia is performed in an extremely time-critical and high-risk setting, where delayed or inaccurate assessment may lead to severe perioperative complications. Patients often present with hemodynamic instability, limited preoperative information, and complex comorbidities, creating major challenges for anesthesiologists to rapidly evaluate airway, circulation, and physiological reserve. Although multiple assessment tools exist, conventional approaches remain fragmented and insufficient for dynamic, real-time decision-making in emergency contexts. There is a clear need for an integrated rapid anesthesia assessment framework that combines advanced monitoring, intelligent decision support, and team-based coordination. This perspective article systematically analyzes rapid assessment models that integrate multimodal point-of-care ultrasound, artificial intelligence–assisted regulation, and closed-loop clinical decision support system (CDSS) protocols. Key applications in perioperative airway management, hemodynamic and respiratory assessment, and risk stratification for special populations, including elderly and pediatric patients, are discussed. We further summarize the rapid titration and hemodynamic advantages of novel anesthetic agents in emergency induction, the role of CDSS and closed-loop communication in clinical emergency management, and the optimization of team collaboration using standardized tools such as Situation–Background–Assessment–Recommendation. Ethical and legal considerations, including informed consent, presumed consent, and proxy decision-making in time-sensitive scenarios, are also addressed. Overall, integrated rapid assessment strategies supported by technology, interdisciplinary teamwork, and ethical safeguards may substantially enhance the safety, efficiency, and clinical outcomes of anesthesia management in emergency surgery, providing a practical framework for future perioperative practice and multicenter validation.

Keywords: Emergency surgery anesthesia, Rapid assessment, Clinical emergency management, Point-of-care ultrasound, Decision support systems, Team collaboration

1 INTRODUCTION

Emergency surgery is often performed under extreme time pressure and marked physiological uncertainty, making it a high-stakes environment for anesthesiologists. Patients frequently present with hemodynamic instability, limited preoperative data, and multiple comorbidities, all of which require anesthesiologists to make rapid yet accurate decisions. While airway predictors such as Modified Mallampati Grading and ultrasound-based measurements (e.g., skin-to-hyoid and skin-

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to-thyroid distances) are commonly used in elective surgeries, their routine application in emergency settings is often impractical due to time constraints and the urgency of the situation [1].

In these high-pressure conditions, effective anesthesia management depends not only on individual expertise but also on strong interdisciplinary coordination. However, communication barriers, cognitive conflicts, hierarchical structures, and resource constraints frequently undermine team performance. Delays or failures in anesthesia induction, airway control, or circulatory stabilization can lead to catastrophic outcomes, underscoring the critical need for efficient and precise management in emergency scenarios. Consequently, emergency anesthesia assessment must prioritize speed, accuracy, adaptability, and continuous reassessment, which are essential for supporting safe perioperative decision-making.

A key component of emergency anesthesia assessment is the rapid identification of high-risk conditions, including airway compromise, circulatory failure, and the loss of respiratory or metabolic reserve. A persistent imbalance between oxygen delivery and consumption may lead to oxygen debt, a condition strongly associated with adverse perioperative outcomes [2]. Early recognition of these risks allows anesthesiologists to tailor anesthesia plans to the individual patient's needs and ensure timely intervention.

Recent advances in monitoring technologies have significantly enhanced the scope of emergency anesthesia assessment. For instance, quantitative electroencephalography can be used to estimate anesthetic depth in neonates, and burst suppression patterns can indicate global cerebral dysfunction [3]. Additionally, bedside ultrasound provides a rapid, noninvasive means of evaluating cardiopulmonary function. When combined with artificial intelligence (AI)-assisted decision-support systems that integrate dynamic physiological data and risk stratification, these technologies improve real-time clinical judgment, further enhancing the safety and efficacy of emergency anesthesia management.

2 EMERGENCY SURGERY ANESTHESIA ASSESSMENT

2.1 Rapid assessment models and tools

Conventional assessment methods are often insufficient in emergency surgery due to rapidly evolving clinical conditions. Integrated rapid assessment models combine multimodal monitoring to support timely decision-making. The X-Porte ultrasound system enables multi-angle lung ultrasound to assess pulmonary status and detect atelectasis or pulmonary edema at the bedside. Portable gastric ultrasound using the Perlas method estimates gastric volume and aspiration risk through measurement of gastric cross-sectional area.

Cardiac function and volume status can be evaluated using phased-array and convex probes, while vascular access and vessel patency are assessed with high-frequency linear probes. The DoCare Anesthesia Clinical Information System (Ver5.0) integrates hemodynamic and hemorheological data from positive-pressure blood flow systems, allowing continuous monitoring and trend analysis [4]. Collectively, these tools improve perioperative safety.

2.2 Heart function and respiratory status assessment strategies

Rapid assessment of cardiopulmonary function is critical for determining anesthetic safety. Continuous monitoring of systolic blood pressure, diastolic blood pressure, mean arterial pressure, heart rate, and oxygenation provides an initial evaluation of circulatory and respiratory status. Airway risk assessment can be refined by integrating anatomical features, airway scores, and medical history.

Ventilation effectiveness is evaluated using tidal volume measurements during initial mask ventilation attempts, followed by continuous monitoring of CO_2 waveforms after airway device placement. Classification of waveform patterns (V1-V3) assists in validating ventilation quality and detecting airway obstruction [5]. This structured approach reduces perioperative risk and supports evidence-based anesthesia management.

2.3 Innovations in assessment for special populations (e.g., elderly, children)

Elderly patients exhibit reduced physiological reserve and altered pharmacokinetics, necessitating careful assessment of cardiovascular, respiratory, and renal function. Cognitive and psychological status can be evaluated using tools such as the 3-Minute Diagnostic Interview for Confusion Assessment Method, the Mini-Mental State Examination, and the Hospital Anxiety and Depression Scale, combined with hemodynamic monitoring to optimize perioperative management [6].

Pediatric emergency anesthesia is complicated by higher heart rates, lower blood pressure, and immature autonomic regulation. Novel anesthetic depth monitors enable continuous assessment of oxygenation and peripheral perfusion, contributing to safer and more precise anesthesia control in time-limited settings.

3 CLINICAL EMERGENCY MANAGEMENT STRATEGIES AND TECHNIQUES

3.1 Application of rapid decision support systems

Clinical decision support systems (CDSS) integrate patient history and real-time physiological data to enhance anesthesia

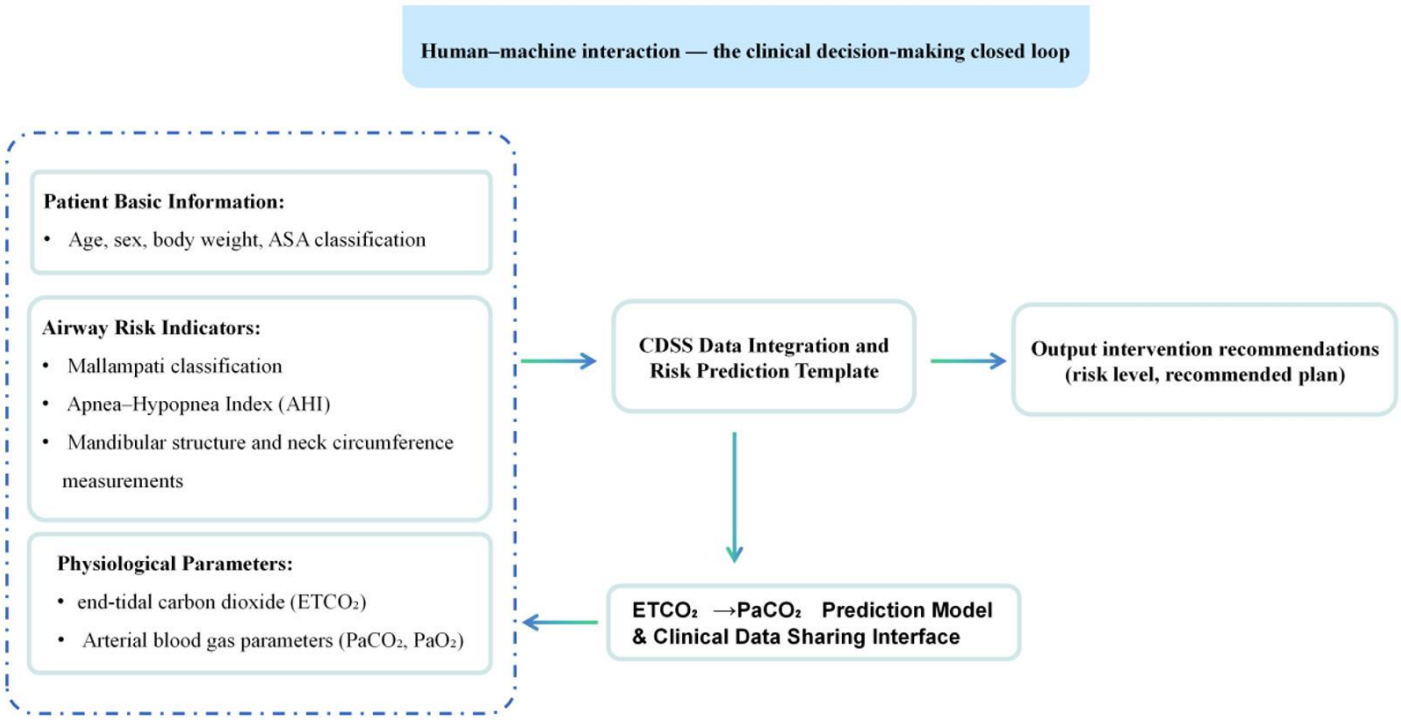

Figure 1. Human-machine interaction–clinical decision making feedback loop. This interface incorporates basic patient information (age, sex, weight, ASA grade), airway risk score (Mallampati classification and disease anatomic profile comprising the AHI, mandibular length, and neck circumference measurements), and physiological data: $ETCO_2$, arterial blood gas parameters including $PaCO_2$ and PaO_2. Once data have been entered into the CDSS integration database and risk prediction template, recommendations (risk category and recommended action) are produced. Feedback updates are realized through the $ETCO_2$–$PaCO_2$ prediction model and clinical presenting interface, constituting an intelligent clinical decision-making feedback cycle. ASA, American Society of Anesthesiologists; AHI, Apnea-Hypopnea Index; $ETCO_2$, end-tidal carbon dioxide; $PaCO_2$, partial pressure of arterial carbon dioxide; PaO_2, partial pressure of arterial oxygen; CDSS, clinical decision support system.

assessment. Models incorporating age, Mallampati score, and respiratory indices can predict difficult mask ventilation. Continuous monitoring of vital signs enables early detection of instability and timely intervention.

Advanced CDSS platforms further employ end-tidal carbon dioxide–based algorithms to estimate arterial carbon dioxide levels, improving ventilation management [7]. Closed-loop systems integrate data acquisition, risk prediction, and feedback mechanisms, enabling dynamic adjustment of anesthesia strategies. These technologies enhance interdisciplinary collaboration and support complex decision-making in emergency settings (see **Figure 1**).

3.2 The rapid application and adjustment of novel anesthetic drugs

In emergency surgery, rapid titration and hemodynamic stability are essential. Newer anesthetic agents such as ciprofol demonstrate reduced cardiovascular variability and allow precise control of sedation depth. The use of intranasal or intravenous dexmedetomidine in combination regimens offers stable hemodynamics and improved recovery profiles in short or high-risk procedures [8]. These agents represent important advances in emergency anesthesia pharmacology. Supplementary Table 1 provides a comprehensive comparative analysis of various anesthetic drugs.

3.3 Optimization of team collaboration and communication in emergency situations

Effective teamwork is fundamental to emergency anesthesia management. Closed-loop communication improves task execution and reduces errors, particularly in high-stress environments. Standardized communication tools such as Situation–Background–Assessment–Recommendation provide a structured framework for information exchange among anesthesiologists, surgeons, and nursing staff. Situation–Background–Assessment–Recommendation enhances clarity, reduces miscommunication, and supports rapid decision-making. Interdisciplinary simulation training further strengthens team coordination and protocol adherence [9].

4 ETHICAL AND LEGAL CONSIDERATIONS IN PRACTICE

4.1 Informed consent and patient rights in emergency situations

Emergency anesthesia frequently involves patients without decision-making capacity, particularly during airway emergencies. When immediate intervention is required to prevent harm, presumed consent may be ethically justified. In such cases, clinicians should document clinical urgency and decision rationale while involving proxy decision-makers whenever feasible [10].

4.2 Legal responsibility and protection of healthcare workers

During emergency airway management, anesthesiologists must balance rapid action with respect for patient autonomy. Practical strategies include brief verbal explanations, proxy consent from family members, and post-event disclosure once the patient stabilizes. Hospitals should establish clear protocols for presumed consent and proxy decision-making to enhance transparency and legal protection [11].

4.3 Ethical conflicts and solutions in emergency decision-making

Ethical decision-making should emphasize beneficence, proportionality, and team-based judgment. Structured documentation and institutional support systems are essential to protect both patient rights and healthcare providers in high-risk scenarios [12].

5 DISCUSSION

This perspective article synthesizes current evidence on rapid anesthesia assessment and clinical emergency management, emphasizing the integration of bedside ultrasound, AI-assisted monitoring, and CDSS-based decision support. These technologies collectively address the limitations of fragmented traditional assessments and enable real-time, patient-specific risk stratification.

Despite promising advances, several limitations remain. Most integrated assessment models lack large-scale clinical validation, and evidence is often derived from single-center or observational studies. Interoperability between monitoring systems and CDSS platforms also remains a technical challenge. Furthermore, the implementation of AI-driven tools requires clinician training and institutional support to avoid overreliance on automated recommendations.

Future research should prioritize multicenter prospective studies to evaluate clinical outcomes, cost-effectiveness, and workflow integration. Standardized protocols combining technology, pharmacological innovation, and team communication are needed to ensure reproducibility and scalability.

In conclusion, rapid anesthesia assessment supported by technological integration, interdisciplinary collaboration, and ethical safeguards represents a transformative approach to emergency surgery. With continued validation and refinement, intelligent anesthesia decision-making systems may become a cornerstone of safe and effective emergency perioperative care.

DECLARATIONS

Author contributions

Songxiao Yang contributed to the conception and design of the study, data collection, analysis, and interpretation, and was responsible for drafting the manuscript; and Ping Zhou revised it critically for important intellectual content.

Funding

This project was supported by the National Natural Science Foundation of China [82260474, 82303935], the Hainan Province Key Research and Development Program [ZDYF2022SHFZ132, ZDYF2024SHFZ045], and the Hainan Province Clinical Medical Center Construction Project.

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.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

Not applicable.

Supplementary Information

The online version (of this article) contains supplementary material available at https://doi.org/10.61189/891717grkkpo.

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Supplementary Information

Supplementary Table 1. Characteristics of new anesthetic drugs in emergency surgery for rapid induction and sedation

Drug name	Main pharmacological features	Monitoring indicators	Hemodynamic effects	Applicable population	Clinical advantages and limitations	References
Etomidate	Emergency endotracheal intubation	Blood pressure, heart rate, SOFA score	Less risk of hemodynamic collapse	Required intubation in critically ill patients	Hemodynamic stability, but associated with lower 7-day survival rate	[1]
Dexmedetomidine	Sedation for mechanical ventilation in sepsis; Early sedation in ICU patients with msTBI; Sedation during weaning from mechanical ventilation	RASS score, CPOT score; ICP monitoring; RASS score and target sedation range	Hypotension, bradycardia	Mechanical ventilation in adult sepsis; TBI patients requiring ICP monitoring; Critically ill patients on mechanical ventilation	Comparable to propofol in efficacy, similar safety profile; Improved outcomes in ICP subgroup, but with hemodynamic risks; Faster extubation, reduced fentanyl requirement, lower incidence of delirium	[2-4]
Propranolol	Sedative sparing in critically ill patients on mechanical ventilation	Changes in sedative dosage and RASS score	Fewer bradycardia events	Critically ill patients requiring high-dose sedatives	Reduced sedative dosage, lower costs; limitations due to open-label design	[5]
Alfentanil	Emergency rapid sequence induction	Systolic blood pressure, heart rate, oxygen saturation	No significant difference in blood pressure and heart rate, with narrower range of variability	Hemodynamic stability in emergency intubation patients	Advantage in reducing blood pressure variability, limitation of small sample size	[6]
Remimazolam	Induction of general anesthesia for surgery	Blood pressure, heart rate, BIS	Smaller decrease in blood pressure, low incidence of hypotension	Patients on angiotensin axis blockers	Low risk of hypotension, availability of antagonists; higher BIS values	[7]
Isoflurane	Sedation for mechanical ventilation in ICU; ICU sedation after cardiac arrest	End-expiratory concentration and sedation depth; Blood pressure and neurological signs monitoring	No significant differences in heart rate and MAP; Vasodilation requiring vasopressors	ICU patients on mechanical ventilation; Post-cardiac arrest resuscitation patients	Promotes spontaneous breathing but concerns about the environment; Opioid-sparing but increased need for vasopressors	[8, 9]
ciprofol	Sedation for mechanical ventilation in ICU	RASS score, plasma concentration	Minimal blood pressure and heart rate fluctuations	ICU intubated patients	Comparable efficacy at lower doses, limited by small sample size	[10]
Propofol sodium phosphate	Deep sedation for mechanical ventilation in ICU	RASS score, Narcotrend index	Hypotension is common but manageable	Patients requiring deep sedation in ICU	Good water solubility, minimal effect on lipids; small sample size	[11]
Ketofol	Rapid induction for emergency laparotomy	Mean arterial pressure, heart rate	Low incidence of hypotension at 1:1 ratio	Adult patients undergoing emergency surgery	Hemodynamic stability; single-center study	[12]
Fentanyl	Pre-treatment for emergency RSI	Blood pressure, heart rate monitoring	Increased risk of hypotension post-intubation	Emergency RSI in adult patients	Attenuates blood pressure increase but increases risk of hypotension	[13]
Rocuronium bromide	Emergency rapid sequence induction	GCS score and blood pressure monitoring	Blood pressure drop after intubation	Adult emergency patients	Risk of inadequate sedation can be mitigated	[14]

Note: SOFA, Sequential Organ Failure Assessment; ICU, intensive care unit; msTBI, mild-to-severe traumatic brain injury; RASS, Richmond Agitation–Sedation Scale; CPOT, Critical-Care Pain Observation Tool; ICP, intracranial pressure; TBI, traumatic brain injury; BIS, Bispectral Index; MAP, mean arterial pressure; RSI, rapid sequence induction; GCS, Glasgow Coma Scale.

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Perioperative Precision Medicine

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PERSPECTIVE

A new era of intelligent decision support in the perioperative period: Innovative applications of artificial intelligence, big data, and large language models

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Received December 21, 2025; Accepted March 12, 2026; Published March 31, 2026

DOI: 10.61189/319254ilybgm

Abstract

Perioperative management is essential for ensuring surgical safety and improving patient outcomes. With increasing surgical complexity and patient heterogeneity, traditional experience-based decision-making is no longer sufficient to meet the demands of precision medicine. Recent advances in artificial intelligence (AI), big data analytics, and large language models (LLMs) provide new opportunities to transform perioperative clinical decision support. AI-driven models enable accurate risk stratification and prediction of perioperative complications, while big data technologies facilitate multimodal data integration, real-time monitoring, and personalized intervention strategies. Meanwhile, LLMs enhance clinical communication, support medical documentation, and assist knowledge-based clinical decision-making through advanced natural language processing capabilities. However, despite rapid technological development, a comprehensive framework integrating AI, big data, and LLMs for perioperative intelligent decision-making remains insufficiently explored. This article reviews emerging applications of AI in preoperative risk assessment, intelligent anesthesia management, and postoperative complication prediction, and summarizes the role of big data in integrated clinical platforms and personalized treatment strategies. It also highlights the potential of LLMs in patient education, clinical decision support systems, and automated knowledge synthesis. Overall, integrating AI, big data, and LLMs may establish an interpretable closed-loop perioperative decision-support ecosystem that improves surgical safety, clinical efficiency, and personalized healthcare delivery.

Keywords: Perioperative management, Artificial intelligence, Big data, Large language model, Clinical decision support, Personalized medicine

1 INTRODUCTION

Perioperative management encompasses preoperative evaluation, intraoperative care, and postoperative recovery. Its primary goal is to reduce surgical risks and complications while improving overall patient outcomes. Effective perioperative management is therefore essential for enhancing surgical safety, optimizing anesthetic strategies, and improving patient satisfaction. However, with the increasing complexity of surgical procedures and the growing heterogeneity of patient populations, traditional experience-based decision-making is becoming insufficient to meet the requirements of modern precision medicine.

Recent advances in artificial intelligence (AI) and big data analytics are transforming perioperative decision support. By integrating multi-source clinical data—including medical history, imaging results, laboratory examinations, and physiological

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monitoring data—machine learning (ML) and deep learning models can assist clinicians in predicting intraoperative complications, postoperative infections, and organ dysfunctions with improved accuracy. Compared with conventional statistical or manually assisted models, AI-based frameworks demonstrate greater sensitivity, dynamic learning capacity, and adaptability, thereby supporting more precise perioperative risk assessment, anesthesia management, and postoperative monitoring.

In addition, the rapid development of large language models (LLMs) has further expanded the potential of intelligent clinical decision support. With strong capabilities in natural language understanding and knowledge reasoning, LLMs can generate patient-specific educational materials, provide evidence-based clinical recommendations, and facilitate doctor–patient communication and medical documentation. Recent studies have shown that ChatGPT-assisted communication tools can significantly reduce preoperative anxiety and improve patient satisfaction, highlighting the growing potential of human–AI collaboration in perioperative healthcare [1].

2 APPLICATION OF AI IN THE PERIOPERATIVE PERIOD

2.1 Preoperative risk assessment and predictive modeling

AI has demonstrated substantial potential in perioperative medicine, particularly in risk prediction, anesthesia management, and postoperative monitoring. In preoperative risk assessment, ML and deep learning models can integrate multimodal clinical data—including medical history, laboratory results, imaging findings, and vital signs—to predict intraoperative complications, postoperative infections, and organ dysfunction with improved accuracy [2]. Compared with conventional statistical approaches, AI-based models provide enhanced sensitivity and dynamic learning capacity for risk stratification. Natural language processing (NLP) can further facilitate automated extraction of information from electronic medical records (EMRs), improving data utilization and reducing human error.

2.2 Intelligent anesthesia management systems

In intraoperative management, intelligent anesthesia systems use ML algorithms to analyze physiological monitoring data and optimize anesthetic depth, analgesia intensity, and hemodynamic stability. Studies have shown that AI-assisted systems can shorten stabilization time, reduce bispectral index fluctuations, and decrease propofol consumption during complex surgeries. Advanced approaches such as extreme gradient boosting and fuzzy control logic also contribute to precise anesthetic management and improved outcome prediction [3].

2.3 Postoperative complication monitoring and intervention

For postoperative care, AI models integrating EMRs and physiological parameters enable early detection of complications such as infection, hemorrhage, and respiratory failure. ML-based models combined with interpretability methods such as Shapley Additive explanations have shown effectiveness in predicting postoperative complications and guiding personalized perioperative interventions [4].

3 THE ROLE OF BIG DATA IN PERIOPERATIVE DECISION-MAKING

3.1 Data integration and analysis platforms

Big data technologies play a fundamental role in perioperative decision support by enabling the integration and real-time sharing of heterogeneous clinical information, including EMRs, physiological monitoring data, imaging results, laboratory findings, and surgical records. Such integrated data platforms provide essential support for preoperative risk assessment, intraoperative management, and postoperative surveillance. By combining structured and unstructured clinical data, these platforms can improve data accessibility, enhance analytical efficiency, and facilitate more accurate and timely clinical decision-making. In addition, clinical decision support systems (CDSS) built on large-scale perioperative data can assist in the early identification of adverse events and postoperative complications. For example, CDSS-based models have been applied to the automatic detection and staging of acute kidney injury, enabling timely alerts and more appropriate interventions [5]. Therefore, the construction of standardized and interoperable perioperative data integration platforms is a key step toward improving the intelligence, efficiency, and reliability of perioperative management.

3.2 Real-time monitoring and feedback

Big data–driven monitoring systems allow real-time analysis of vital signs, anesthesia indicators, and intraoperative parameters, enabling the early warning of abnormal physiological states. Such systems improve perioperative safety and decision accuracy. For instance, ankle pump motion monitoring devices provide real-time feedback to support venous thromboembolism prevention and improve patient compliance. Wearable devices have also been shown to enhance postoperative activity levels and relieve dyspnea in lung cancer surgery patients [6].

3.3 Personalized treatment strategies

Big data further supports personalized perioperative medicine by integrating genomic, imaging, and clinical information. Systems such as the Intelligent Perioperative System can pre-

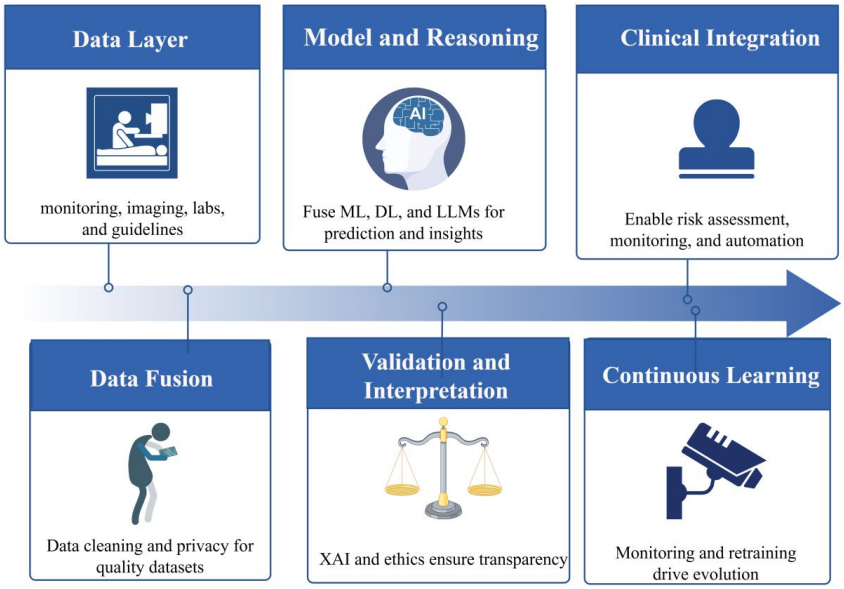

Figure 1. Framework of the Perioperative Intelligent Decision Support System (Created with Biorender). This figure demonstrates the global architecture of the perioperative intelligent decision support system and includes six layers, i.e., data layer, data fusion layer, model and reasoning layer, validation and interpretation layer, clinical integration layer and continuous learning layer. It serves as a platform that merges multimodal data sources from monitoring, imaging, and laboratories to apply ML, deep learning and LLMs for risk prediction and intelligent reasoning. Explainable AI and ethical review processes are embedded to guarantee model transparency and accountability. During clinical integration, the system includes components for risk assessment and automatic decision-making, and incorporates a continuous learning and retraining process for self-optimization of the DSS over time. ML, machine learning; DL, deep learning; LLMs, large language models; XAI, explainable artificial intelligence; DSS, decision support system; AI, artificial intelligence.

dict surgical complications and generate decision support using high-throughput data processing technologies. Advanced monitoring tools, including brain oxygenation monitoring systems such as Galileo, provide real-time cerebral perfusion and oxygenation data to guide individualized perioperative management (as shown in Supplementary Table 1) [7].

4 APPLICATION PROSPECTS OF LANGUAGE MODELS

4.1 Preoperative patient education and communication

LLMs can improve preoperative education and communication by generating personalized materials based on patient characteristics and clinical data, helping patients better understand surgical procedures, risks, and precautions. Their natural language interaction supports multilingual communication and enhances clinician–patient engagement. Studies show that ChatGPT-assisted informed consent can reduce perioperative anxiety and improve satisfaction among patients undergoing total knee arthroplasty [8]. However, variations in accuracy and language complexity may limit comprehension for patients with low health literacy, indicating the need for further validation and optimization in medical communication.

4.2 Intelligent CDSS

LLMs are rapidly advancing the intelligence of CDSS. By analyzing EMRs, imaging data, and real-time monitoring information, LLMs can provide evidence-based diagnostic and therapeutic recommendations, supporting faster and more accurate clinical decisions. Systems such as ChatENT demonstrate advantages in medical education and clinical decision support. Techniques such as Structured Query Rewriting further enhance automation and platform independence in clinical decision-making, supporting more intelligent perioperative management (see **Figure 1**) [9].

4.3 NLP in literature review

NLP enables automated literature screening, topic clustering, and evidence extraction, improving the efficiency and objectivity of systematic reviews. NLP-based living systematic reviews allow real-time updates of emerging evidence. Deep learning models such as BioBERT have shown strong performance in abstract screening, with potential to partially replace manual review processes and improve consistency in systematic reviews [10].

5 DISCUSSION AND FUTURE PERSPECTIVES

Although big data, AI, and LLMs have shown considerable promise in perioperative decision support, several important challenges remain before these technologies can be widely implemented in routine clinical practice. Current systems still face limitations related to data heterogeneity, model generalizability, interpretability, privacy protection, and ethical governance. In particular, multimodal data integration may increase the risk of privacy leakage and algorithmic bias, highlighting the need for more robust regulatory and technical safeguards.

Future research should focus on several specific directions. First, multicenter prospective studies are needed to validate the performance, robustness, and clinical utility of AI- and LLM-based perioperative decision-support systems across different surgical populations and healthcare settings. Second, more attention should be given to the development of interpretable models that can provide transparent reasoning for risk prediction, treatment recommendation, and intraoperative decision support. Third, privacy-preserving methods, such as differential privacy, federated learning, and secure multiparty compu-

tation, should be further integrated into perioperative data platforms to balance data security with model performance. Fourth, future systems should aim to establish closed-loop intelligent frameworks linking preoperative assessment, intraoperative monitoring, and postoperative outcome analysis to support continuous learning and dynamic optimization. In addition, recent studies have suggested that generative AI–assisted clinical systems may improve physicians' decision-making consistency and efficiency in perioperative care. Overall, the next generation of perioperative intelligent decision-support systems should be developed through close collaboration among clinicians, data scientists, engineers, and ethicists, with the goal of achieving safe, interpretable, and clinically applicable intelligent healthcare systems.

ABBREVIATIONS

BioBERT, Biomedical Bidirectional Encoder Representations from Transformers; ChatENT, Augmented Large Language Model for Expert Knowledge Retrieval in Otolaryngology-Head and Neck Surgery; EHR, electronic health records; LLM, Large Language Model; LLMs, large language models; ML, Machine Learning.

DECLARATIONS

Author contributions

Di Wang and Shafinah Ahmad Suhaimi contributed to the conception, writing, revision, and final approval of the manuscript. Both authors have read and approved the final version of the manuscript.

Funding

This research received no external funding.

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.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

Not applicable.

Supplementary Information

The online version (of this article) contains supplementary material available at https://doi.org/10.61189/319254ilybgm.

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Supplementary Information

Supplementary Table 1. Typical applications and outcomes of big data in perioperative management

Data type	Application scenario	Analytical method	Key findings and clinical significance	References
Preoperative EHR	Postoperative delirium risk prediction	ML and logistic regression	The predictive performance of the ML model is significantly superior to that of traditional methods	[1]
Provincial-level medical administrative data	Prognostic prediction for emergency surgery in older adults	Comparative analysis of multiple frailty assessment models	Frailty indices significantly improve the accuracy of mortality risk prediction	[2]
Multicenter standardized EHR	Cardiac risk prediction for non-cardiac surgery	Multiple ML algorithms	ML models demonstrate significantly superior predictive performance compared to traditional scoring systems	[3]
Multi-surgery EHR	Patient risk stratification and phenotype identification	Clustering and random forest algorithms	Digital phenotyping significantly enhances postoperative risk prediction	[4]
EHR of cancer patients	Prediction of severe postoperative complications	Interpretable ML model	Accurate prediction of complications and interpretation of risk factors	[5]
Joint replacement registry and EHR data	Prediction of PJI risk	Multiple ML and traditional models	Limited improvement in model performance; clinical utility remains questionable	[6]
Preoperative electronic health record data	Postoperative in-hospital mortality risk prediction	Random forest ML	Automated risk scoring significantly outperforms traditional methods	[7]
EHR and ICD coding	Automated mortality risk scoring for pediatric surgery	Algorithmic automation and statistical comparison	Enables automated risk stratification and reduces clinical workload	[8]
Perioperative EHR	Prediction of scute kidney injury after vascular surgery	Random forest ML	Incorporating intraoperative data significantly improves predictive performance	[9]
Electronic health record data	Preoperative frailty assessment in elderly surgical patients	Comparative performance analysis of multiple frailty assessment tools	Electronic frailty tools significantly enhance risk stratification	[10]
Continuous invasive blood pressure data	Prediction of intraoperative hypotension	ML algorithm	Significantly reduces the duration and depth of hypotension	[11]
Continuous invasive arterial pressure data	Quantification of intraoperative hypotension severity	Multivariable proportional odds model	Invasive monitoring significantly improves the detection rate of hypotension	[12]
Clinical, body composition, and CT imaging data	Preoperative prediction of postoperative pancreatic fistula risk	Integration of ML and deep learning	Development of a high-precision preoperative prediction model	[13]
CT imaging and clinical data	Preoperative prediction of postoperative pancreatic fistula risk	Multivariate logistic regression	Construction of a high-accuracy predictive nomogram model	[14]
Panoramic radiograph	Prediction of third molar extraction difficulty and risk	Semantic segmentation and classification model	Construction of a high-precision preoperative decision-support system	[15]

Note: EHR, electronic health records; ML, machine learning; PJI, periprosthetic joint infection; ICD, International Classification of Diseases; CT, computed tomography.

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Perioperative Precision Medicine

Zentime

REVIEW ARTICLE

Mechanisms of traditional Chinese medicine extracts in ameliorating sepsis-induced myocardial injury

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Received September 5, 2025; Accepted March 3, 2026; Published March 31, 2026

DOI: 10.61189/873111rbsjxe

Abstract

Sepsis-related myocardial dysfunction significantly increases patient mortality and remains a major challenge in critical care medicine. Its pathological mechanisms involve uncontrolled inflammation, excessive oxidative stress, and impaired stress tolerance of cardiomyocytes. Currently, no specific treatment methods are available. Although supportive treatment remains the mainstream in clinical practice, effective interventions for septic myocardial injury are still lacking. This review systematically summarizes the cardioprotective effects of traditional Chinese medicine (TCM) preparations, including Xuebijing injection (containing safflower, red peony root, and *salvia miltiorrhiza*) and Shenfu Injection (containing ginseng and aconite), in cell and animal models. The mechanisms include antioxidation, anti-inflammation, and enhancement of cardiomyocyte resistance to injury. Some of these preparations have been recommended in the "Guidelines for the Diagnosis and Treatment of Sepsis" in China and are used as adjunctive treatments in intensive care units (ICUs). Preliminary clinical studies suggest that these agents may improve microcirculation and potentially enhance survival rates. This review not only highlights the multi-target potential of TCM in sepsis-induced myocardial injury but also provides a direction for future high-quality mechanistic research and international multicenter randomized controlled trials, thereby facilitating the integration of traditional medicine into modern critical care practice.

Keywords: Sepsis-induced myocardial injury, Traditional Chinese medicine extracts, Anti-inflammatory agents, Antioxidants, Herbal cardioprotection, Clinical trials

Highlights

- This review presents a comprehensive overview of the pharmacological effects of traditional Chinese medicine (TCM) in sepsis-induced myocardial injury.
- It systematically summarizes the molecular mechanisms and recent research advancements regarding protective effects of TCM in sepsis.
- It proposes a novel "temporal treatment" strategy for Perioperative Sepsis, aligning TCM interventions with the dynamic pathophysiological stages of the disease.

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1 INTRODUCTION

Sepsis is a life-threatening syndrome triggered by dysregulated host immune response to infection and is characterized by uncontrolled systemic inflammation and progressive multi-organ dysfunction. The heart, as a highly metabolically active and richly perfused organ, is particularly vulnerable to injury during sepsis. Clinical data show that approximately 40%-50% of critically ill patients develop detectable myocardial injury, manifested by decreased left ventricular ejection fraction and reduced cardiac output among others, significantly prolonging ICU stay and increasing mortality risk [1-5]. The pathological mechanisms of sepsis are complex and multifactorial, involving excessive pro-inflammatory factor release, mitochondrial oxidative stress, abnormal calcium acc umulation, and impaired myocardial energy metabolism, all of which jointly lead to inhibited myocardial contractility [6-10].

Although significant progress has been made in anti-infection, fluid resuscitation, and organ support treatments, there are still no specific drugs that can directly target sepsis-induced myocardial damage. This unmet clinical need has prompted growing interest in therapeutic strategies with pleiotropic properties, including anti-inflammatory, antioxidative, and cardioprotective effects. Traditional Chinese Medicine (TCM) has demonstrated potential value in this context. Over thousands of years, TCM has accumulated clinical experience in managing acute and severe syndromes that similar to modern sepsis, such as “heat toxins” and “warm diseases” [11-14]. More importantly, TCM emphasizes the “holistic view” and “diagnosis-based treatment”, achieving systemic regulation through the synergistic effect of multiple components, multiple targets, and multiple pathways, which precisely aligns with the high heterogeneity and network-based pathological characteristics of sepsis [15-20].

In recent years, a large number of high-quality preclinical studies have provided mechanistic evidence supporting the cardioprotective effects of TCM. For instance, baicalin from *Scutellaria baicalensis* and paeonol from *Paeonia lactiflora* have been proven to simultaneously inhibit the NF-κB-mediated inflammatory cascade and activate the Nrf2/HO-1 antioxidant pathway, thereby attenuating cardiomyocyte apoptosis and functional impairment [21-28]. Furthermore, the application of modern technologies, including network pharmacology, transcriptomics, and gene-edited animal models, has facilitated the transformation of traditional empirical prescriptions into evidence-based therapies with increasingly defined molecular mechanisms [29, 30]. Notably, the core TCM principle of “strengthening vital qi and eliminating pathogenic factors while harmonizing yin and yang” is highly consistent with the current international trend of sepsis management, which is evolving from simple pathogen eradication toward reconstruction of immune homeostasis and organ function maintenance [31-33].

Given the increasingly solid experimental and preliminary clinical evidence supporting the myocardial protective effects of TCM in sepsis, a systematic synthesis of current findings is urgently needed. This review aims to comprehensively summarize recent advances in TCM-derived extracts and compound preparations in this field, with particular emphasis on their bioactive components, molecular targets, signaling pathways, and translational potential [34, 35].

The structure of this review is as follows. First, the core pathophysiology of septic cardiomyopathy is briefly outlined. Second, single herbs and active compounds with reliable preclinical evidence (e.g., Scutellaria baicalensis, Salvia miltiorrhiza, and ginsenosides) and their cardioprotective mechanisms are systematically reviewed. Third, the efficacy and safety of clinically applied TCM injections (e.g., Xuebijing injection and Shenfu injection) are analyzed. Finally, key challenges—including compositional complexity, insufficient quality standardization, and limited high-level clinical evidence—are discussed, and future research directions are proposed to promote the scientific, standardized, and internationalized development of TCM in modern critical care medicine (**Figure 1**).

2 RESEARCH PROGRESS ON THE MECHANISMS BY WHICH TCM EXTRACTS IMPROVE SEPSIS-INDUCED MYOCARDIAL INJURY

In recent years, substantial progress has been made in elucidating the mechanisms by which TCM extracts ameliorate sepsis-induced myocardial injury. Several preclinical studies have shown that multiple bioactive components derived from TCM can effectively suppress excessive inflammatory responses, eliminate reactive oxygen species (ROS), improve mitochondrial function, and regulate cardiomyocyte energy metabolism through multi-target and multi-pathway modulation, thereby exerting cardioprotective effects [36-38]. These mechanisms not only provide biological support for the holistic framework of TCM, but also offer mechanistic insights and potential candidate compounds for the development of novel cardioprotective strategies. This section systematically reviews the mechanisms of action of representative TCM extracts and summarizes the corresponding experimental evidence, laying the foundation for a deeper understanding of their therapeutic potential.

2.1 Pathological mechanisms of sepsis-induced myocardial injury

Sepsis is characterized by dysregulated host response to infection, leading to multiple organ dysfunction and high mortality [39]. Among the various organ complications, sepsis-induced myocardial injury represents a clinically significant manifestation that contributes substantially to disease severity and mortality [40]. The pathogenesis of sepsis-induced myocardial injury is multifactorial and involves a complex interplay of

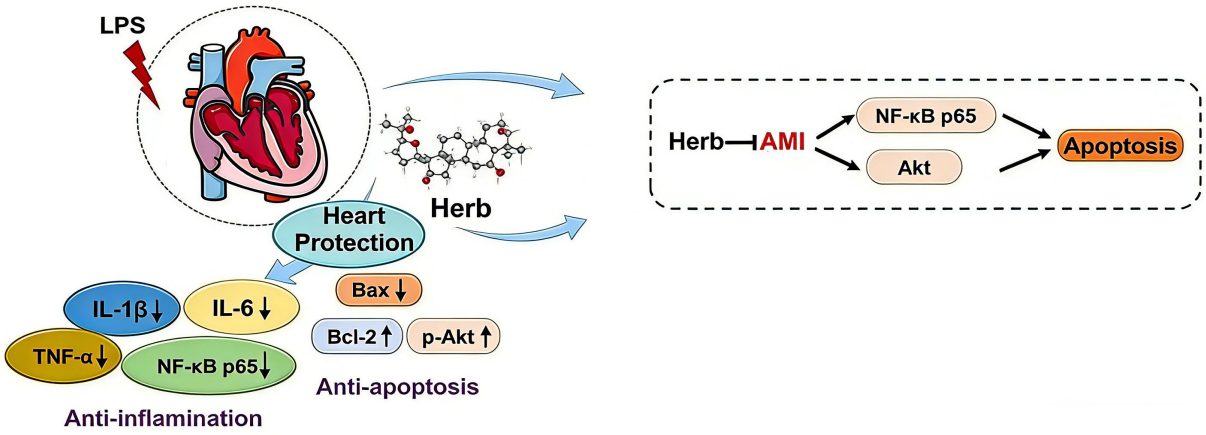

Figure 1. The mechanism of the effect of TCM extracts on improving myocardial injury caused by sepsis. Herbal intervention (labeled "Herb") exerts protective effects on the heart following insult by lipopolysaccharide (LPS) or other AMI-inducing stimuli. The herb modulates key signaling pathways to suppress inflammation and apoptosis. Specifically, it downregulates pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) and inhibits NF-κB p65 activation, thereby attenuating inflammatory responses. Concurrently, the herb promotes cell survival by activating the Akt pathway (increasing p-Akt), upregulating anti-apoptotic Bcl-2, and downregulating pro-apoptotic Bax. These coordinated actions converge to inhibit apoptotic cell death via suppression of NF-κB p65 and Akt-mediated signaling cascades. Overall, this multi-targeted mechanism contributes to myocardial protection in the context of acute cardiac injury. The figure created with Microsoft PowerPoint 2019. AMI, acute sepsis-induced myocardial injury.

inflammatory responses, oxidative stress, and cardiomyocyte apoptosis [41, 42]. A comprehensive understanding of these mechanisms is essential for the development of effective therapeutic strategies to alleviate sepsis-related cardiac dysfunction.

2.1.1 Association between inflammatory responses and myocardial injury

Excessive inflammatory activation represents a major mechanism underlying sepsis-induced myocardial injury, characterized by the massive release of pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, and IL-6 [43-47]. While these mediators are essential for pathogen clearance, their excessive production exerts opposite effects on cardiac tissue [48]. The increase in these inflammatory cytokines are associated with myocardial dysfunction, decreased cardiomyocyte contractility, and increased susceptibility to apoptosis, all of which contribute to cardiac dysfunction [49].

Nuclear factor-κB (NF-κB) plays a crucial role in mediating sepsis-related cardiac injury. Upon activation, it induces the transcription of numerous pro-inflammatory genes, thereby amplifying cytokine production and sustaining the inflammatory cascade, which directly impairs cardiomyocyte function and promotes structural damage. Experimental studies have shown that inhibition of NF-κB pathway signaling attenuates myocardial injury and protects cardiac function in sepsis models, highlighting this pathway as a potential therapeutic target [50]. In addition to systemic cytokine release, during sepsis, activated immune cells (especially macrophages) accumulate in cardiac tissue and secrete additional inflammatory mediators, thereby intensifying local inflammation and contributing to myocardial dysfunction [51, 52].

The interplay between inflammation and myocardial injury is further amplified by oxidative stress. Excessive inflammatory activation promotes the generation of ROS, which disrupt cellular homeostasis and damage lipids, proteins, and nucleic acids within cardiomyocytes [53, 54]. Importantly, oxidative stress further enhances inflammatory signaling, forming a vicious cycle. In sepsis, this vicious cycle contributes to progressive cardiac dysfunction. Therefore, targeted modulation of inflammatory responses represents a critical strategy for interrupting this pathogenic loop and protecting myocardial function.

2.1.2 Role of oxidative stress in sepsis-induced myocardial injury

Oxidative stress is a critical mechanism contributing to myocardial injury during sepsis, characterized by excessive ROS production and insufficient antioxidant capacity. Upon activation, neutrophils and macrophages generate substantial amounts of ROS as part of the host immune response [55, 56]. When ROS production exceeds the endogenous antioxidant

capacity, oxidative damage to cardiomyocytes ensues. Due to the high metabolic demand and dense mitochondrial content, myocardial tissue is particularly susceptible to oxidative injury. Excessive ROS attack cellular lipids, proteins and DNA, leading to mitochondrial dysfunction, disruption of calcium homeostasis, and activation of cardiomyocyte death pathways [57, 58]. In addition, ROS can activate the NLRP3 inflammasome, thereby promoting the release of inflammatory factors and further aggravating the inflammatory response, establishing a vicious cycle of "oxidative stress - inflammation" [59-61]. Therefore, attenuation of oxidative stress may help interrupt this pathogenic cascade, protect cardiac function, and improve clinical outcomes.

Antioxidant therapy has been proposed as a promising approach to mitigate oxidative injury in sepsis. Early studies in cellular and animal models have shown that antioxidants like N-acetylcysteine (NAC) can effectively reduce ROS accumulation, attenuate myocardial injury, and improve cardiac function [62, 63]. However, clinical translation yielded inconsistent results. Some clinical trials have failed to demonstrate significant benefits, suggesting that antioxidant therapy may not be universally effective. Factors such as timing of application, dosage, and disease severity, and patient heterogeneity likely influence therapeutic efficacy.

2.1.3 Mechanisms of myocardial cell apoptosis and injury

Cell apoptosis is a highly regulated process of programmed cell death. In sepsis-induced myocardial injury, apoptosis plays a critical role: a physiological mechanism for eliminating damaged or old cells is exploited by the disease, ultimately contributing to myocardial damage.

In sepsis, myocardial cells are not simply overwhelmed by inflammation or toxins; rather, intrinsic cellular processes are disrupted. These disruptions include signaling pathway alterations, mitochondrial dysfunction, activation of death-associated proteins. It's like an "insurrection" initiated from within, gradually leading the cells towards self-destruction. The seemingly quiet apoptosis actually exacerbates myocardial damage in an insidious manner. Inflammatory cytokines, oxidative stress, and mitochondrial dysfunction act synergistically to trigger apoptotic signaling pathways [64, 65]. Both intrinsic and extrinsic apoptotic pathways are mobilized, leading to the sequential activation of caspase, which orchestrate the degradation of cellular architecture, ultimately leading to the irreversible loss of functional cardiomyocytes [66, 67].

At the molecular front line, the balance between pro-survival and pro-apoptotic signals is tightly regulated by proteins such as Bax and Bcl-2. In the context of sepsis, this equilibrium is disrupted, with Bax, a pro- apoptotic protein, prevailing over Bcl-2, the key anti-apoptotic protein, resulting in an elevated Bax/Bcl-2 ratio that promotes widespread cardiomyocyte death [68, 69]. In addition, the NF-κB pathway, which is centrally involved in inflammation, further amplifies myocardial cell apoptosis by upregulating the expression of pro-apoptotic genes, thereby contributing to myocardial injury beyond its role in immune activation [70].

The strategic inhibition of apoptosis has therefore emerged as a compelling avenue for cardiac protection in sepsis. Preclinical investigations have shown that targeting caspase activation or reinforcing mitochondrial resilience can significantly reduce cardiomyocyte death and preserve cardiac function under septic conditions [71, 72]. However, despite these advances, clinical translation remains elusive.

In conclusion, sepsis-induced myocardial injury is not the result of a single factor but rather a complex interplay between inflammation, oxidative stress, and cell apoptosis. These factors act synergistically, exacerbating each other's effects and contributing to the progressive decline in cardiac function.

2.2 Mechanisms of action of TCM extracts

TCM has been practiced for centuries, emphasizing holistic management rather than targeting individual symptoms. Nowadays, many studies have demonstrated the efficacy of TCM extracts in eliminating free radicals, slowing down cellular aging, and reducing inflammation, rather than simply masking the symptoms [73-75]. More impressively, some of these extracts have demonstrated cardioprotective effects, such as improving blood circulation, stabilizing blood pressure, and protecting myocardial cells from injury. Commonly used herbs like *Salvia miltiorrhiza*, *Astragalus membranaceus*, and *Panax notoginseng* have long been recognized by modern medicine for their cardiovascular benefits [76-78].

Sepsis-induced myocardial injury (SIMI) is a life-threatening complication of systemic infection, characterized by reversible biventricular dilation, reduced ejection fraction, and elevated cardiac biomarkers—despite adequate fluid resuscitation. Unlike ischemic cardiomyopathy, SIMI is not caused by coronary occlusion, but rather by a cascade of biochemical insults (e.g., excessive ROS, uncontrolled inflammatory cascades, mitochondrial dysfunction, and programmed cell death). Conventional supportive therapies often fail to address these intertwined mechanisms, creating an urgent need for multi-target interventions. In this context, TCM extracts—long used in East Asia for "qi deficiency" and "blood stasis" syndromes resembling circulatory collapse—are gaining increasing scientific attention. These extracts are shown to simultaneously modulate oxidative, inflammatory, and apoptotic pathways with remarkable precision.

2.2.1 Targeting oxidative stress pathways

Oxidative stress is a foundational driver of SIMI. During sepsis, activated neutrophils and dysfunctional mitochondria pro-

duce excessive ROS (e.g., superoxide, hydrogen peroxide), while antioxidant defenses—such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx)—become overwhelmed and depleted. This redox imbalance results in oxidative damage of lipids, proteins, and DNA, impairing myocardial contractility and initiating downstream injury cascades.

TCM herbs counteract oxidative stress through a dual strategy: direct free radical scavenging and, more importantly, transcriptional upregulation of endogenous antioxidant systems. For example, *Astragalus membranaceus* (Huang Qi) and *Panax ginseng* (Ren Shen) have been shown to enhance SOD and GPx activity, accelerating ROS clearance, and reducing malondialdehyde (MDA)—a marker of lipid peroxidation—in both animal models and clinical cohorts [79, 80]. Notably, their effects extend far beyond enzymatic support. For instance, *Astragalus polysaccharides* (APS) exerts a cardioprotective effect by targeting the endoplasmic reticulum (ER) stress signaling pathway to inhibit cardiomyocyte apoptosis in diabetic cardiomyopathy. APS downregulates the phosphorylation level of protein kinase R-like ER kinase (PERK) and inhibits the oligomerization and autophosphorylation of inositol-requiring enzyme 1α (IRE1α), thereby blocking two critical ER stress signaling cascades (PERK-eIF2α-ATF4 and IRE1α-XBP1-CHOP). Meanwhile, this regulatory effect reduces the activation of Caspase-12, a specific apoptotic protease in ER stress-induced apoptosis and blocks its cross-talk with the mitochondrial apoptotic pathway, providing a clear molecular basis for APS's application in perioperative myocardial protection for diabetic patients [81].

The core regulator of this adaptive response is nuclear factor erythroid 2–related factor 2 (Nrf2). Normally sequestered in the cytoplasm by Keap1 and targeted for degradation, Nrf2 is liberated upon oxidative challenge or pharmacological stimulation. Once translocated to the nucleus, it binds to antioxidant response elements (AREs), driving the expression of over 200 cytoprotective genes—including HO-1, NQO1, and glutathione synthetase—that collectively enhance cellular resilience to oxidative stress [82, 83]. *Salvia miltiorrhiza* (Danshen), a cornerstone herb in cardiovascular TCM formulas, activates Nrf2 via its diterpenoid (tanshinones) and phenolic acid (salvianolic acids) constituents, which not only scavenge free radicals but also preserve membrane integrity by inhibiting lipid peroxidation [84, 85].

Remarkably, some TCM compounds achieve target specificity comparable to that of synthetic drugs. For instance, hyperoside—a flavonoid derived from *Hypericum perforatum*—though not classical TCM herb but increasingly studied in integrative contexts—binds covalently to cysteine residues on Keap1, preventing Nrf2 ubiquitination and enabling sustained nuclear accumulation. This structurally precise interference with protein–protein interactions reveals a level of mechanistic sophistication once thought exclusive to small-molecule pharmaceuticals [86-88]. Thus, TCM extracts function not merely as passive antioxidants but as dynamic "redox rheostats", recalibrating cellular defense architecture in real time.

2.2.2 Targeting inflammatory signaling pathways

While inflammation is essential for pathogen clearance, its dysregulation in sepsis leads to a self-amplifying cytokine storm that directly depresses myocardial contractility through mechanisms such as nitric oxide–mediated calcium desensitization and β-adrenergic uncoupling. Rather than broadly immunosuppressing—a strategy that increases the risk of secondary infections, TCM emphasizes restoring immune homeostasis, which aligns with the classical TCM principle of "harmonizing the defensive qi".

Two key inflammatory signaling pathways are targeted by TCM: NF-κB and the NLRP3 inflammasome. NF-κB, often referred to as the "master switch" of immune responses, regulates the transcription of TNF-α, IL-6, IL-1β, and adhesion molecules. TCM interventions selectively modulate these pathways, applying molecular brakes without impairing host defense. For instance, *Scutellaria barbata* (Ban Zhi Lian) inhibits IκB kinase (IKK), preventing IκB degradation and subsequent NF-κB nuclear translocation, thereby dampening inflammatory mediator production [89]. Gossypin, a flavonoid derived from *Hibiscus* species and structurally similar to several TCM flavonoids, exerts dual control by suppressing both cytokine mRNA stability and NF-κB activation, positioning it as a candidate for precision immunomodulation in hyperinflammatory states [90, 91].

In addition to NF-κB, the NLRP3 inflammasome plays a critical role in sepsis-induced inflammation. It senses damage-associated molecular patterns (DAMPs) and catalyzes the caspase-1–dependent maturation of IL-1β and IL-18. The clinically approved *Xuebijing* injection, a five-herb formula (Carthamus, Paeonia, Ligusticum, Salvia, Angelica) has demonstrated efficacy in randomized controlled trials (RCTs) for sepsis, significantly reducing serum IL-1β levels and improving cardiac index [92]. Its active components, such as hydroxysafflor yellow A and ferulic acid, inhibit NLRP3 oligomerization and ASC speck formation, effectively disrupting "disconnecting" the inflammasome's activation and subsequent inflammatory amplification.

Beyond cytokine suppression, TCM also reprograms the innate immune response. Macrophages, key mediators of cardiac inflammation, can polarize into either a pro-inflammatory M1 phenotype (producing TNF-α, ROS) or a reparative M2 phenotype (secreting IL-10 and TGF-β) [93, 94]. Quercitrin—a flavonoid abundant in *Rhus chinensis* and other TCM herbs—shifts macrophage polarization toward M2 phenotype via STAT6

activation, transforming the cardiac microenvironment from destructive to regenerative [95]. This functional plasticity reflects a higher-order strategy: not only blocking mediators but also reshaping the immune landscape to favor tissue resolution and repair.

2.2.3 Targeting apoptosis and mitochondrial dysfunction

Cardiomyocyte apoptosis, though limited in absolute number, disproportionately impairs cardiac output due to the post-mitotic nature of heart cells. In sepsis, apoptosis is primarily triggered by ROS, TNF-α, and calcium overload, which converge on mitochondrial outer membrane permeabilization (MOMP) and caspase activation. Consequently, preserving mitochondrial integrity is paramount.

TCM extracts protect mitochondria through multiple mechanisms. Ginsenoside R1, a protopanaxatriol saponin from red ginseng, activates AMP-activated protein kinase (AMPK), a central energy sensor that enhances mitochondrial biogenesis (via PGC-1α), promotes mitochondrial fusion via Mfn2 and stimulates mitophagy to remove damaged organelles. This multifaceted action alleviates cardiac lipotoxicity and prevents the release of cytochrome c, a key event in apoptosis [96]. Clinically, *Shenmai* injection (red ginseng + Ophiopogon japonicus) has been shown to reduce troponin levels and improves left ventricular ejection fraction in patients receiving anthracycline chemotherapy or suffering from septic cardiomyopathy, partly by modulating Bcl-2/Bax ratios and inhibiting caspase-3 cleavage [97, 98].

Guanxin Ning injection (Salvia miltiorrhiza+Ligusticum chuanxiong) has demonstrated robust protection against ischemia–reperfusion injury—a model highly relevant to SIMI—by enhancing electron transport chain efficiency (Complexes I and III), stabilizing mitochondrial membrane potential, and delaying mitochondrial permeability transition pore (mPTP) opening [99]. Moreover, several TCM formulations have been shown to promote angiogenesis via VEGF/PI3K/Akt signaling. Xuebijing injection, for instance, increases capillary density in peri-infarct zones and improves myocardial perfusion, thereby mitigating hypoxia-driven apoptosis and supporting functional recovery [100, 101].

Critically, the effects of these TCM extracts are synergistic: by reducing oxidative stress, TCM limits NF-κB/NLRP3 activation; by tempering inflammation, it preserves mitochondrial function; and by stabilizing mitochondrial integrity, it blocks intrinsic apoptosis—thereby enhancing tissue repair. This systems-level action mirrors the TCM diagnostic framework, where "deficiency" (e.g., qi deficiency) and "excess" (e.g., heat-toxin) coexist and must be addressed concurrently.

2.3 Related research progress

2.3.1 Research in animal models

Recent animal experiments have provided valuable insights into the potential of TCM in treating sepsis. For instance, *Bai Pu Da* (BBD) showed significant therapeutic effects in mice with sepsis caused by lipopolysaccharide (LPS): not only prolonged the survival but also significantly reduced inflammatory factors such as IL-1β and TNF-α [102]. This suggests that BBD may help stabilize excessive inflammation and reduce organ damage.

Further studies have shown that MLG, extracted from chrysanthemum, showed efficacy in a cecal ligation and puncture (CLP) sepsis model, which better mimics human septic conditions. MLG not only inhibited the inflammatory response in macrophages but also increased the survival rate of mice. Its effectiveness is attributed to its ability to "switch off" two key inflammatory signaling pathways, TAK1-NF-κB and MAPK, thereby reducing systemic inflammation and organ damage [103].

Furthermore, "Xingbi Jing" (XBJ), which has been approved for use in treating sepsis in China, has also been found to alleviate acute lung injury in rats caused by sepsis. This therapeutic effect was achieved by regulating oxidative stress and inflammatory responses, which are key contributors to the high mortality associated with sepsis [104].

Overall, these animal experiments not only enhance our understanding of the mechanisms through which TCM exerts its therapeutic effects but also highlight its potential in clinical applications. Without these models, the mechanism of TCM might remain partially understood rather than fully elucidated.

2.3.2 Preliminary results of the clinical trial

A systematic review of multiple RCTs has indicated that TCM interventions, decoction or injection, can significantly improve inflammatory markers and renal function in patients with sepsis with concomitant kidney damage [105].

For instance, the well-known "Xuebijing" injection has been reported to improve the renal microcirculation of patients with sepsis, while reducing inflammatory factors that exacerbate the inflammatory cascade. These findings suggests that *Xuebijing* may serve as a reliable alternative therapy apart from antibiotics [106]. The key outcome indicators of Xuebijing injection in perioperative sepsis were shows in **Table 1**.

Additionally, a compound preparation named *Hongrun Injection*, which contains several herbal ingredients, has been

Table 1. Key outcome indicators of Xuebijing injection in perioperative sepsis

Indicator category	Specific indicator	Effect size (mean difference/odds ratio)	95% confidence interval (CI)	*P* Value	Effect direction (role of Xuebijing)	Reference
Microcirculation parameters	Mean arterial pressure (MAP)	+4.00 mmHg	[1.20, 6.80]	<0.05	Improvement (Increased)	[107, 108]
	Perfused small vessel density (PVD)	+6.43 mm/mm^2	[4.15, 8.71]	<0.01	Improvement (Increased)	
	Microvascular flow index (MFI)	0.61	[0.48, 0.74]	<0.01	Improvement (Increased)	
Oxygenation status	Pimonidazole adduct (Hypoxia)	-35.20% (Staining Area)	[-48.60, -21.80]	<0.05	Improvement (Decreased)	[109]
Inflammatory factors	Interleukin-1β (IL-1β)	-28.60 pg/mL	[-39.40, -17.80]	<0.05	Inhibition (Decreased)	[110-112]
	Interleukin-6 (IL-6)	-34.10 pg/mL	[-45.70, -22.50]	<0.05	Inhibition (Decreased)	
	Tumor necrosis factor-α (TNF-α)	-21.30 pg/mL	[-30.90, -11.70]	<0.05	Inhibition (Decreased)	
	High mobility group box 1 (HMGB-1)	-19.80 pg/mL	[-28.50, -11.10]	<0.05	Inhibition (Decreased)	
Kidney injury biomarkers	Urinary TIMP-2*IGFBP-7	-1.80 ng/mL	[-2.50, -1.10]	<0.05	Protection (Decreased)	[113, 114]
	Urinary NGAL	-42.70 ng/mL	[-56.30, -29.10]	<0.05	Protection (Decreased)	
	Serum creatinine (SCr)	-83.40 μmol/L	[-112.60, -54.20]	<0.01	Protection (Decreased)	
Histopathology	Paller score (Renal Tubular Injury)	-12.70 Points	[-16.90, -8.50]	<0.01	Protection (Decreased)	[115]
survival status	7-Day survival rate	+0.02 (Odds Ratio)	[0.85, 1.19]	>0.05	No significant effect	[116]

Note: MAP, Mean arterial pressure; PVD, Perfused small vessel density; MFI, Microvascular flow index; IL-1β, Interleukin-1β; IL-6, Interleukin-6; TNF-α, Tumor necrosis factor-α; HMGB-1, High mobility group box 1; TIMP-2, Tissue inhibitor of metalloproteinase 2; IGFBP-7, Insulin-like growth factor-binding protein 7; NGAL, Neutrophil gelatinase-associated lipocalin; SCr, Serum creatinine.

shown to significantly reduce the mortality rate in patients with sepsis compared to those receiving conventional treatment alone [117].

2.3.3 Comparative study of different Chinese medicinal herbal extracts

Various Chinese medicinal herbs possess distinct therapeutic "specialties" and modes of action when dealing with sepsis. For example, the extract of *Sophora flavescens* demonstrated a potent anti-inflammatory effect in an LPS-induced sepsis model. It not only outperformed other herbal remedies but also significantly reduced the levels of pro-inflammatory factors such as TNF-α and IL-6 [118]. On the other hand, the polysaccharides of *Ganoderma lucidum* take a different approach: they not only enhance immune function but also reduces oxidative damage, showing promising potential in sepsis management [119].

Take two commonly used TCM injections in clinical practice for example: BBD mainly "extinguishes the fire" by inhibiting the NLRP3 inflammasome; while XBJ protects organs and reduces damage by regulating ferroptosis and alleviating oxidative stress.

These examples clearly demonstrate that TCM treatments are not a "single formula that can cure all diseases". Instead, each herbal extract has its own mechanism and distinct advantages. To optimize the use of TCM in treating sepsis, it is crucial to understand the specific therapeutic targets of each component and how their combination can be optimized for better results.

2.4 Application prospects of TCM extracts in sepsis treatment

2.4.1 Challenges in clinical application

Although TCM extracts hold considerable promise for the treatment of sepsis, several challenges remain before they can be widely applied in clinical practice. Sepsis is an inherently complex condition—once the immune system becomes dysregulated, systemic inflammation and organ failure ensue, resulting in high mortality rate [120]. While TCM has several centuries of experience in treating infections, translating these experiences into therapies recognized by modern medicine remains a significant hurdle [121].

One major obstacle is the lack of standardization in TCM. Different manufacturers produce varying formulations, often with significant differences in batches, extraction methods, and even the chemical composition of the same herbal remedy, preventing the establishment of consistent, reliable data on the efficacy and safety of these treatments.

The scientific community generally relies on high-quality evidence, especially RCTs. However, most TCM prescriptions consist of multiple ingredients, each targeting various molecular pathways, emphasizing holistic regulation. This is fundamentally different from the "one drug, one target" paradigm of Western medicine. Therefore, applying the RCT methodology to evaluate TCM is extremely challenging. This has resulted in a rather awkward situation: although many clinicians and patients believe in the efficacy of TCM, the lack of solid data, commonly available for Western medicine, makes it difficult to

substantiate its benefits with scientific evidence [122, 123]. Furthermore, the integration of TCM into sepsis treatment requires collaboration between Western medicine and TCM practitioners. However, due to their differing approaches, communication and collaboration remain difficult.

The complexity of sepsis itself adds another layer of difficulty, as TCM extracts often exert multiple effects, such as anti-inflammatory, antioxidant, and immune regulation. Although this is an advantage, it also complicates identification of specific components responsible for therapeutic effects. Questions arise about potential interactions with antibiotics or other conventional treatments [124, 125].

Therefore, more rigorous and comprehensive research is needed in the future. Not only should we investigate whether TCM can improve patient outcomes, but we also need to determine the precise mechanisms through which TCM works and how it can be combined with existing treatments. Only by solving these problems can TCM truly fulfill its potential in the treatment of sepsis and provide broader benefits to patients.

2.4.2 Future research directions

For TCM extracts to truly "establish a firm foothold" in the treatment of sepsis, research efforts should be directed towards several key areas.

First of all, we need to employ more robust scientific methodologies to elucidate how TCM extracts work. For instance, modern technologies such as molecular biology, network pharmacology, and high-throughput screening can help identify the active TCM ingredients and determine which specific targets they modulate within the immune system [126-128]. Only by understanding the mechanism can we explain clearly how TCM regulates inflammation, enhances immunity, and protects organs - rather than simply stating "it works", without being able to provide a rationale.

Second, the extraction and formulation of TCM must be standardized. A major challenge currently is the variability in the effects of different batches of herbal medicines, which significantly hinders their clinical promotion. Therefore, it is essential to establish unified and stable production processes and quality control standards [129]. Furthermore, clear medication guidelines should be formulated, including dosage and administration timing, to support the conduct of high-quality clinical trials.

Regarding experimental research, future studies should focus on designing rigorous RCTs, especially to evaluate the combined effects of TCM extracts and existing therapies (such as antibiotics and fluid resuscitation). It is possible that TCM could generate a synergistic effect when used in conjunction with conventional treatments [130]. Additionally, the timing of administration and optimal dosage are crucial aspects that need to be addressed through systematic research.

Safety considerations are equally important. TCM extracts may interact with Western medications, potentially affecting their metabolism or elimination. In critical conditions such as sepsis, extra caution is necessary. Therefore, pharmacokinetic and pharmacodynamic profiles of TCM extracts must to be specifically evaluated, and adverse reactions should be closely monitored in clinical settings [131].

In addition, beyond focusing solely on treating diseases, it is also worth exploring whether TCM can actually prevent diseases. TCM has long emphasized "preventing diseases before they occur". In high-risk populations for sepsis (such as patients with severe infections), could the use of certain TCM extracts serve as a preventive measure, or act as an auxiliary intervention in the early stages of the disease? This could potentially reduce the risk of progression and warrants further exploration [132].

In conclusion, the integration of ancestral wisdom with modern scientific research methods is crucial. By fostering interdisciplinary collaboration and respecting the holistic perspective of TCM, while also explaining its mechanisms and clinical value in scientific language, TCM extracts can truly be integrated into the modern treatment system for sepsis.

2.4.3 Exploration of comprehensive treatment strategies

Combining TCM extracts with conventional treatments may offer a particularly promising approach to improving the management of sepsis [133]. After all, sepsis is not a single issue but rather a complex interplay of issues, including immune dysfunction, inflammatory storms, and organ damage. Addressing this multifaceted condition with a single medication is challenging. However, TCM's emphasis on holistic conditioning can precisely fill this gap.

Many studies have shown that TCM herbal extracts are effective in regulating the immune system, suppressing excessive inflammation, and protecting organs. These properties make them particularly suitable as adjunctive treatment in sepsis management.

A promising approach is to combine TCM extracts with conventional treatments such as antibiotics, fluid resuscitation, and life support. For instance, some TCM formulations not only enhance the efficacy of antibiotics in killing bacteria but also improve microcirculation, reduce inflammation, and alleviate oxidative stress. These combined effects may improve overall treatment outcome and potentially reduce the risk of developing antibiotic resistance.

Table 2. Summary of pharmacological studies on TCM extract

TCM name (latin name)	Active components	Core targets/signaling pathways	Study type	Results	Reference
Astragalus membranaceus	Astragalus polysaccharides (APS), astragaloside IV	Nrf2-ARE, NF-κB, PERK/IRE1α, Bcl-2/Bax	Cell experiment, Animal experiment, clinical RCT	1. Enhances SOD and GPx activities and reduces MDA levels; 2. Inhibits ER stress-mediated cardiomyocyte apoptosis; 3. Improves $CD4^+/CD8^+$ ratio, reduces postoperative sepsis incidence by 35%	[136, 137]
Salvia miltiorrhiza	Tanshinone IIA, salvianolic acid A/B	Nrf2, AMPK, VEGF/PI3K/Akt, NF-κB	Cell experiment, animal experiment, clinical RCT	1. Activates Nrf2 pathway to enhance antioxidant capacity; 2. Promotes mitochondrial biogenesis and autophagy; 3. Improves myocardial perfusion, reduces 90-day readmission rate by 25%	[138, 139]
Scutellaria baicalensis	Baicalin, baicalein	NF-κB, JAK-STAT, NLRP3 inflammasome	Cell experiment, animal experiment, clinical RCT	1. Inhibits cytokine storm (IL-6 and TNF-α reduced by 55%); 2. Stabilizes vascular endothelium and improves MAP; 3. Exhibits synergistic bacteriostatic effect with antibiotics	[140, 141]
Panax ginseng	Ginsenoside R1, Rg1, Rb1	AMPK/PGC-1α, eNOS, Bcl-2/Bax	Cell experiment, animal experiment, clinical RCT	1. Enhances myocardial mitochondrial function and reduces apoptosis; 2. Improves myocardial oxygen consumption, reduces postoperative troponin I level by 40%; 3. Regulates neuro-endocrine-immune network	[142, 143]
Panax notoginseng	Ginsenoside Rg1, Rb1	eNOS, mitochondrial apoptotic pathway	Animal experiment, clinical RCT	1. Increases NO production and protects endothelial function; 2. Alleviates myocardial ischemia-reperfusion injury; 3. Improves myocardial oxygenation in patients undergoing cardiovascular surgery	[144, 145]
Carthamus tinctorius	Hydroxysafflor yellow A	NLRP3, NF-κB	Cell experiment, animal experiment	1. Inhibits NLRP3 inflammasome activation and reduces IL-1β release; 2. Improves microcirculation and reduces vascular permeability	[146, 147]
Xuebijing injection (Compound)	Hydroxysafflor yellow A, ferulic acid, etc.	TLR4/NF-κB, HO-1, coagulation pathway	Clinical RCT, animal experiment	1. Reduces 28-day mortality by 18% and improves SOFA score; 2. Inhibits coagulation disorders and reduces microthrombus formation; 3. Improves renal microcirculation and reduces SCr	[148, 149]
Shenmai injection (Compound)	Ginsenosides, ophiopogon polysaccharides	Bcl-2/Bax, caspase-3	Clinical RCT, animal experiment	1. Reduces troponin level in sepsis patients; 2. Improves left ventricular ejection fraction; 3. Alleviates myocardial mitochondrial damage	[149, 150]

Note: APS, Astragalus polysaccharides; Nrf2-ARE, Nuclear factor erythroid 2-related factor 2-antioxidant response element; NF-κB, Nuclear factor kappa-B; PERK/IRE1α, Protein kinase R-like endoplasmic reticulum kinase/Inositol-requiring enzyme 1α; Bcl-2/Bax, B-cell lymphoma-2/Bcl-2-associated X protein; RCT, Randomized controlled trial; SOD, Superoxide dismutase; GPx, Glutathione peroxidase; MDA, Malondialdehyde; AMPK, Adenosine 5'-monophosphate-activated protein kinase; VEGF/PI3K/Akt, Vascular endothelial growth factor/Phosphatidylinositol 3-kinase/Protein kinase B; JAK-STAT, Janus kinase-signal transducer and activator of transcription; NLRP3, NOD-like receptor thermal protein domain associated protein 3; IL-6/TNF-α, Interleukin-6/Tumor necrosis factor-α; MAP, Mean arterial pressure; PGC-1α, Peroxisome proliferator-activated receptor gamma coactivator 1-α; eNOS, Endothelial nitric oxide synthase; TLR4, Toll-like receptor 4; HO-1, Heme oxygenase-1; SOFA, Sequential Organ Failure Assessment; SCr, Serum creatinine.

Moreover, TCM may help mitigate the side effects of conventional treatments. For instance, antibiotics often cause gastrointestinal damage. Some Chinese herb combinations or interventions (such as acupuncture) may help alleviate gastrointestinal discomfort and maintain intestinal barrier function - an essential consideration for sepsis patients, as their intestines are often damaged [134]. Additionally, therapies focusing on overall condition, such as acupuncture and tonic herbs, can supplement conventional treatments, facilitating faster recovery and improving patients' quality of life.

Another key direction for future research is personalized treatment. TCM has long emphasized "treating each patient individually". By taking into account a patient's background (such as underlying diseases, genetic characteristics, and immune status), and combining this information with the disease stage, the most appropriate combination of TCM and Western medicines could be selected, potentially enhancing treatment precision [135]. This approach necessitates the identification of reliable biomarkers to guide treatment decisions, including when and which TCM intervention to use for optimal results.

In conclusion, integrating TCM extracts into the comprehensive treatment of sepsis should not aim to replace existing therapies, but to achieve a "synergistic effect". The summary of pharmacological studies and basic characteristics of included clinical studies on TCM extract were shown in **Tables 2** and **3**, respectively.

Table 3. Basic characteristics of included clinical studies on TCM extract

Study design	Sample size (intervention/control)	Intervention measures	Control measures	Primary outcomes	Secondary outcomes	References
Multicenter RCT	600/600	Preoperative oral astragalus polysaccharides (100 mg/day) for 2-4 weeks + standard perioperative care	Standard perioperative care (antibiotic prophylaxis, fluid resuscitation)	Incidence of postoperative sepsis (primary); 30-day mortality	ICU length of stay, $CD4^+/CD8^+$ ratio, serum IL-6/TNF-α levels	[151]
Single-center RCT	100/100	Intraoperative Xuebijing injection (20-40 mL/h continuous infusion) + Sepsis Bundle Therapy	Sepsis bundle therapy (early antibiotics, source control, organ support)	28-day mortality (primary); Change in SOFA score at 72 hours	Lactate clearance rate, coagulation parameters (INR, platelet count), renal function (SCr)	[152]
Single-center RCT	40/40	Intraoperative baicalin (100 mg bolus + 50 mg/h maintenance) + conventional therapy	Conventional therapy (antibiotics, vasopressors, fluid resuscitation)	Change in mean arterial pressure (MAP) at 6 hours; Serum IL-6 level reduction	Incidence of septic shock, 14-day mortality, adverse events (hypotension, allergy)	[153]
Single-center RCT	75/75	Preoperative oral panax notoginseng extract (50 mg/day) for 2 weeks + CABG perioperative care	CABG perioperative care (cardiopulmonary bypass management, antiplatelet therapy)	Postoperative troponin I level at 24 hours; Myocardial oxygen consumption	Left ventricular ejection fraction (LVEF), length of hospital stay, surgical site infection rate	[154]
Multicenter RCT	150/150	Postoperative salvia miltiorrhiza Injection (20 mL/day) for 7-10 days + standard rehabilitation	Standard rehabilitation (nutritional support, physical therapy)	90-day readmission rate (primary); LVEF improvement at 1 month	Myocardial fibrosis markers, inflammatory factors (CRP, procalcitonin), quality of life score	[155]
Systematic review + meta-analysis	12 RCTs (n=1,280 total)	Xuebijing injection (100-200 mL/day) + conventional sepsis treatment	Conventional sepsis treatment (antibiotics, renal replacement therapy if needed)	Change in serum creatinine (SCr); Renal microcirculation parameters (PVD, MFI)	Urinary NGAL/TIMP-2*IGFBP-7 levels, Paller score (renal tubular injury), 30-day survival	[156]
Single-center RCT	50/50	Postoperative Shenmai Injection (20 mL/day) for 5-7 days + septic cardiomyopathy management	Septic cardiomyopathy management (inotropic agents, diuretics)	Change in LVEF at 1 week; Serum troponin level reduction	BNP level, myocardial mitochondrial function markers, length of ICU stay	[157]
Meta-analysis	15 RCTs (n=1,500 total)	Postoperative astragalus Injection (20 mL/day) for 5-7 days + standard postoperative care	Standard postoperative care (infection surveillance, wound care)	Infection recurrence rate (primary); Quality of life score	Macrophage phagocytic activity, serum IFN-γ level, adverse events (hyperkalemia)	[158]

Note: RCT, Randomized controlled trial; ICU, Intensive care unit; IL-6, Interleukin-6; TNF-α, Tumor necrosis factor-α; SOFA, Sequential Organ Failure Assessment; INR, International normalized ratio; SCr, Serum creatinine; MAP, Mean arterial pressure; CABG, Coronary artery bypass grafting; LVEF, Left ventricular ejection fraction; CRP, C-reactive protein; PVD, Perfusion vessel density; MFI, Microvascular flow index; NGAL, Neutrophil gelatinase-associated lipocalin; TIMP-2, Tissue inhibitor of metalloproteinase 2; IGFBP-7, Insulin-like growth factor-binding protein 7; BNP, Brain natriuretic peptide; IFN-γ, Interferon-γ.

3 PERIOPERATIVE SEPSIS: EPIDEMIOLOGY, PATHOPHYSIOLOGICAL FEATURES, AND TEMPORAL STRATEGIES FOR TCM INTERVENTIONS

Perioperative sepsis remains a critical global health concern, characterized by high morbidity, mortality, and imposing significant economic burden on healthcare systems. Its development reflects a complex interplay among patient vulnerability, surgical stress responses, and potential microbial exposure. A comprehensive understanding of its epidemiological distribution, underlying pathophysiological cascades, stage-specific therapeutic strategies is essential for improving clinical outcomes. TCM, with its holistic philosophy and emphasis on syndrome differentiation, offers a complementary approach within contemporary perioperative care. This section systematically reviews the epidemiological characteristics of perioperative sepsis, delineates its distinct pathophysiological mechanisms from community-acquired sepsis, and proposes a structured temporal framework for integrating TCM interventions across perioperative phases.

3.1 Epidemiological characteristics of perioperative sepsis

The epidemiology of perioperative sepsis is heterogeneous and involves multiple determinants, including patient-specific factors, surgical characteristics, institutional protocols, and broader socio-economic conditions. Its incidence and clinical impact vary substantially across different contexts, necessitating a multidimensional analytical perspective.

3.1.1 Incidence and variability across surgical specialties

Systematic reviews of global data from the past decade reported an overall incidence of perioperative sepsis ranging from 2% to 15%, though the true figure may be underestimated due to inconsistent diagnostic criteria [159, 160]. The most striking feature is the immense variability across surgical disciplines. High-risk procedures, particularly those involving the gastrointestinal tract, are associated with substantially elevated incidence rates. Abdominal surgeries, including bowel resections, procedures for perforated viscus, and pancreatic operations, frequently report sepsis rates exceeding 10%, and can be as high as 20-30% in complicated or contaminated cases [161, 162]. These elevated rates are attributed to the disruption of colonized luminal organs, significant tissue trauma, prolonged operative duration, and the risk of anastomotic leakage. In contrast, "clean" surgeries such as elective orthopedic procedures (e.g., joint replacements, spinal surgeries) generally exhibit lower sepsis rates, typically below 5%. Nevertheless, the risk is not negligible, particularly in elderly patients or those with multiple comorbidities undergoing prolonged procedures. Cardiothoracic and major vascular surgeries represent an intermediate-risk category, with sepsis rates influenced by factors such as cardiopulmonary bypass time, intraoperative hypothermia, and the implantation of prosthetic material. Neurosurgical procedures, while primarily clean, carry a unique risk related to surgical site infection, which may progress to meningitis or intracranial abscess, potentially progressing to systemic sepsis. This disparity underscores the critical importance of surgical site characteristics and the degree of physiological trespass in determining sepsis risk. The concept of surgical wound classification (clean, clean-contaminated, contaminated, dirty/infected) remains a fundamental, though imperfect, tool for predicting postoperative infectious complications and subsequent sepsis.

3.1.2 Patient-specific risk factors

Beyond surgical factors, patient demographics and baseline physiological reserves are major determinants of sepsis susceptibility.

Advanced age is one of the most consistent and potent risk factors. Age-related immune remodeling, commonly referred to as immunosenescence, is characterized by reduced naive T-cell output, impaired neutrophil function, and a chronic, low-grade pro-inflammatory state ("inflammaging"). This diminishes effective pathogen clearance while simultaneously increasing the risk of a dysregulated, hyperinflammatory response. A large retrospective cohort study involving more than 5,000 surgical patients demonstrated that individuals over 65 years old had a 2.5-fold higher risk of developing perioperative sepsis compared to younger adults [163]. Furthermore, sepsis in the elderly often presents atypically, without classic signs of fever or leukocytosis, leading to delays in diagnosis and treatment, which contributes to poorer clinical outcomes.

Pre-existing chronic diseases significantly amplify sepsis risk. Diabetes mellitus is a prototypical example, with evidence showing approximately a 1.8-fold higher incidence in postoperative sepsis among diabetic patients [164]. The underlying mechanisms are multifactorial: hyperglycemia impairs neutrophil chemotaxis and phagocytosis, induces endothelial dysfunction, and promotes a pro-inflammatory milieu. Additionally, diabetic neuropathy and microangiopathy may delay infection recognition. Chronic obstructive pulmonary disease (COPD) is associated with impaired mucociliary clearance and chronic airway colonization by pathogenic bacteria, thereby increasing the risk of postoperative pneumonia, a common precursor to sepsis. End-stage renal disease contributes to uremia-associated immunosuppression, while chronic liver disease impairs protein synthesis (including coagulation factors and opsonins) and Kupffer cell function. Malignancy, especially in patients receiving cytotoxic chemotherapy, predisposes to bone marrow suppression and neutropenia. The concept of "frailty", a syndrome of decreased physiological reserve across multiple organ systems, is increasingly recognized as a powerful integrative predictor of vulnerability to surgical stress and postoperative complications, including sepsis.

Malnutrition, particularly protein-calorie malnutrition, weakens both cellular and humoral immunity, impairs wound healing, and reduces respiratory muscle strength, thereby increasing vulnerability to pneumonia and surgical site infection. Obesity, on the other hand, is associated with chronic adipose tissue inflammation, technical surgical complexity, higher rates of deep wound infection, and altered antibiotic pharmacokinetics. Chronic alcohol consumption suppresses immune function and increases intestinal permeability, while smoking compromises pulmonary defense mechanisms and tissue oxygenation.

Suboptimal adherence to evidence-based perioperative protocols directly affects sepsis incidence, including delayed or inappropriate administration of surgical antibiotic prophylaxis, breaches in sterile technique, suboptimal perioperative glycemic control, and inappropriate management of invasive devices like urinary catheters and central venous lines, which serve as potential portals for infection. The increasing prevalence of multidrug-resistant organisms (MDROs) within healthcare settings, including methicillin-resistant *Staphylococcus aureus*

and carbapenem-resistant Enterobacteriaceae, has significantly complicated the management of postoperative infections and contributed to increased sepsis-related mortality.

The burden of perioperative sepsis is disproportionately concentrated in low- and middle-income countries. A multi-center study conducted across sub-Saharan Africa reported alarmingly high perioperative sepsis rates ranging from 18 to 22%, with associated mortality rates exceeding 40%, compared to approximately 10-15% in high-income countries [165]. The reasons for these disparities are multifactorial and systemic, including limited access to sterile surgical environments and reliable sterilization equipment, shortages of essential antimicrobial agents, delayed or unavailable microbiological diagnostics, advanced disease severity at presentation due to delayed healthcare access, and fewer critical care capacity for organ support. These disparities highlight that perioperative sepsis represents not only a clinical challenge but also a manifestation of healthcare inequality, demanding targeted interventions at the health system level.

3.1.3 Temporal dynamics of onset

Early-onset sepsis (Within 48 hours) is most commonly linked to intraoperative events. Causes include gross contamination from a perforated viscus, inadequate antisepsis of the surgical field, contamination from operating room personnel or the environment, and—although rare—contamination of intravenous fluids or anesthesia equipment. The rapid onset typically suggests exposure to a high microbial inoculum or infection with highly virulent organisms.

Delayed-onset sepsis (7-14 days postoperatively) is more commonly associated with postoperative complications occurring during the wound-healing phase. The most frequent source is surgical site infection, which may involve the superficial incision layer, deep soft tissues, or an organ/space compartment (e.g., intra-abdominal abscess). Other sources include hospital-acquired pneumonia (often ventilator-associated pneumonia), catheter-associated urinary tract infections, and infections related to intravascular devices. Hematogenous dissemination from a distant and occasionally occult infectious focus is also possible. Recognizing this temporal distribution is essential for optimizing postoperative surveillance, guiding diagnostic evaluation, and informing the selection of empirical antibiotic therapy.

3.2 Pathophysiological mechanisms of perioperative sepsis

The pathophysiology of perioperative sepsis shares fundamental features with sepsis from other etiologies; however, it is distinguished by a planned surgical insult, which precedes and conditions the infectious event. This "two-hit" paradigm, where operative trauma acts upon a potentially vulnerable host, results in a unique and often more unstable clinical trajectory.

3.2.1 The "first hit": surgical trauma

Even in the absence of infection, major surgery induces a profound systemic inflammatory response syndrome. Tissue injury and cellular necrosis leads to the release of endogenous DAMPs, including high-mobility group box 1 (HMGB1), heat shock proteins (HSPs), mitochondrial DNA (mtDNA), and adenosine triphosphate (ATP). These molecules are recognized by pattern recognition receptors (PRRs) on immune cells, such as Toll-like receptors (TLRs) and NOD-like receptors (NLRs). TLR4, for instance, recognizes HMGB1, initiating a signaling cascade that converge on NF-κB, a central transcriptional regulator of pro-inflammatory gene expression, resulting in a massive release of early response cytokines including TNF-α and IL-6. This sterile inflammation priming alters endothelial function and contributes to postoperative organ dysfunction. Simultaneously, the surgical stress response, mediated by activation of the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system, results in elevated circulating cortisol and catecholamines, which have complex, often immunosuppressive effects in the later phases.

3.2.2 Microbial invasion and the "second hit"

In perioperative sepsis, the sterile inflammatory "first hit" is rapidly amplified by a "second hit" of pathogenic invasion. Unlike community-acquired sepsis, commonly originating from pneumonia or UTI, the infectious source in perioperative sepsis is directly related to the operative site or perioperative complications. Microbial components, such as LPS from Gram-negative bacteria and lipoteichoic acid from Gram-positive bacteria, act as pathogen-associated molecular patterns (PAMPs), providing a potent second signal through PRRs, thereby exponentially amplifying the inflammatory cascade. A critical and often underrecognized factor is the disruption of the gut barrier. Surgical stress, perioperative ischemia–reperfusion injury, opioid use, and alterations in mesenteric perfusion increase intestinal permeability, allowing translocation of bacteria and endotoxins from the gut lumen into the systemic circulation and lymphatic system. This not only provides a source of pathogens but also intensifies systemic inflammation, creating a vicious cycle.

3.2.3 The unique role of coagulation dysregulation

The convergence of surgical trauma and sepsis creates a prothrombotic milieu, predisposing patients to profound hemostatic disturbance. Surgery inherently induces endothelial injury, resulting in exposure of tissue factor (TF) and activation of the extrinsic coagulation pathway. Platelets are activated by both damaged endothelium and inflammatory cytokines. Sepsis further exacerbates coagulation abnormalities by causing widespread endothelial dysfunction, suppressing natural anticoagulant pathways (e.g., protein C, antithrombin III), and impairing fibrinolysis. The cumulative effect results in a shift

toward a hypercoagulable state, manifested as disseminated intravascular coagulation (DIC) in its early pro-thrombotic phase. Microvascular thrombosis develops in vital organs (e.g., lungs and kidneys), contributing to tissue hypoxia and multiple organ dysfunction syndrome (MODS). This thrombotic tendency is often more pronounced in perioperative sepsis compared to medical sepsis. The histopathological study of patients with sepsis revealed that in surgical sepsis cases, there were diffuse microthrombi in the lung and kidney tissues, while in non-surgical sepsis cases, this proportion significantly decreased. This highlights the synergistic pro-coagulant effect of tissue damage and systemic inflammation [166].

3.2.4 Myocardial dysfunction in the perioperative context

Sepsis-induced myocardial dysfunction, often termed septic cardiomyopathy, presents a particular challenge in postoperative patient. It is characterized by biventricular dilatation, reduced left ventricular ejection fraction (LVEF), and attenuated responsiveness to fluid resuscitation and catecholamines. In the perioperative setting, septic cardiomyopathy occurs against a background of additional cardiac stressors, including intraoperative myocardial ischemia–reperfusion injury (particularly in cardiac surgery), fluid shifts, perioperative blood loss, and the increased metabolic demands associated with wound healing and systemic inflammation. Echocardiographic studies reveal that patients undergoing perioperative sepsis may experience acute reductions in LVEF of 20-30%. While often reversible within 7-10 days following effective infection control, acute deterioration can precipitate circulatory collapse [167]. The mechanisms are multifactorial and include circulating myocardial depressant factors (e.g., TNF-α and IL-1β), direct bacterial toxin effects, mitochondrial dysfunction with impaired energy production, disruption of intracellular calcium handling, and excessive production of nitric oxide leading to pathological vasodilation and direct cardiotoxicity.

3.2.5 The immunological paradox and sepsis-induced immunosuppression

Following the initial hyperinflammatory phase, many sepsis patients enter a state of sustained immunosuppression, which is increasingly recognized as a major driver of late morbidity and mortality due to secondary infections. This immunosuppressive phenotype is characterized by widespread lymphocyte apoptosis (particularly affecting B cells and $CD4^+$ T helper cells), expansion of immunosuppressive regulatory T cells and myeloid-derived suppressor cells (MDSCs), and monocyte deactivation (reduced HLA-DR expression). In perioperative patient, this immunosuppressive phase may be further exacerbated by anesthesia-related immunomodulation, blood transfusion, and postoperative pain management. Collectively, these factors predispose patients to delayed-onset nosocomial infections and complicating postoperative recovery.

3.3 Temporal framework for TCM interventions in perioperative sepsis

A distinctive strength of TCM lies in its dynamic, stage-based therapeutic framework, aligning with the evolving pathophysiological trajectory of perioperative sepsis. The principle of "treatment according to temporal stage" (Yishi Zhifa) provides a structured paradigm for tailoring interventions across the perioperative continuum, with interventions directed toward the dominant pathological patterns at each phase.

3.3.1 Preoperative phase (prevention): immune priming and metabolic conditioning

The TCM principle of "treating the undiseased" (Zhi Weibing) parallels the modern strategy of prehabilitation. The goal is to strengthen Zhengqi (upright qi, or host defensive/adaptive capacity) to better withstand the upcoming surgical trauma (Xieqi or pathogenic factor). This involves fortifying the immune system, stabilizing metabolic homeostasis, and improving organ functional reserve.

Astragalus membranaceus (Huang Qi) is regarded as the foundational herb for preoperative fortification. Its bioactive constituents—APS and saponins (astragalosides)—exhibit remarkable immunomodulatory properties. APS can enhance the phagocytic activity of macrophages, promote lymphocyte proliferation and differentiation, and modulates the Th1/Th2 balance toward immune homeostasis. APS has also been reported to increase regulatory T cells, which may help modulate the postoperative inflammatory response and prevent excessive tissue damage. Furthermore, Astragalus exhibits anti-inflammatory effects by inhibiting the NF-κB pathway, thereby reducing the production of TNF-α, IL-6, and other pro-inflammatory cytokines. Several meta-analyses of randomized controlled trials, primarily conducted in China, have indicated that preoperative supplementation with Astragalus (typically for 1-4 weeks) may significantly reduce the incidence of postoperative infectious complications (including sepsis) by approximately 30-40% and shorten hospital stays in patients undergoing major surgery [168].

Panax notoginseng (San Qi), renowned for its ability to invigorate blood and resolve stasis without increasing hemorrhagic risk, is particularly suitable for preoperative microcirculatory optimization. Its principal ginsenosides (Rg1, Rb1) exert antioxidant effects, scavenging ROS generated during ischemia-reperfusion injury. These compounds also protect endothelial function by promoting nitric oxide release and reducing endothelial adhesion molecule expression. A study demonstrated that preoperative administration of Panax notoginseng significantly reduced postoperative myocardial injury biomarkers, with troponin I levels approximately 40% lower than controls [169]. Improvements in indices of myocardial oxygen utiliza-

tion were also observed, suggesting cardioprotection mediated through mitochondrial stabilization and suppression of apoptosis-related signaling pathways [170, 171].

Effective implementation of preoperative TCM conditioning requires adequate lead time. Clinical guidelines and studies suggest initiating preoperative TCM conditioning 2-4 weeks before elective major surgery to allow measurable modulation of immune parameters and metabolic parameters. A dose-response study on APS indicated that the preoperative optimization approach should be personalized based on TCM pattern diagnosis [172]. For example, supplementing Qi and Yin may be appropriate for frail elderly patient characterized by Qi-Yin Deficiency, while clearing damp-heat and strengthening the spleen may be indicated in patient with chronic gastrointestinal dysfunction.

3.3.2 Intraoperative and immediate postoperative phase (hyperinflammatory stage): anti-inflammatory and organ protection

This phase corresponds to the peak of sterile and infection-driven systemic inflammatory responses and early development of organ dysfunction. In TCM theory, this stage is often characterized as *Heat Toxin Blazing Internally* (Redu Neisheng), accompanied by Qi Stagnation and blood stasis, and in severe cases, impending Yang collapse. Accordingly, the TCM strategy shifts to clearing heat, detoxifying, cooling the blood, and protecting organ function, while cautiously preserving Zhengqi.

Herbs like *Scutellaria baicalensis* (Huang Qin), Forsythia suspensa (Lian Qiao), and *Isatis indigotica* (Ban Lan Gen) have strong antibacterial and endotoxin-neutralizing properties. Baicalein, derived from Scutellaria, inhibits pro-inflammatory enzymes such as cyclooxygenase-2 (COX-2) and lipoxygenase (LOX) and suppresses NF-κB activation, thereby attenuating transcription of TNF-α, IL-6, and related cytokines. Salvia miltiorrhiza (Dan Shen) and Carthamus tinctorius (Hong Hua) are particularly relevant in addressing sepsis-associated microcirculatory dysfunction and DIC. Tanshinones derived from Salvia improve blood flow, inhibit platelet aggregation, and exert endothelial antioxidant effects, thereby mitigating thromboinflammatory injury.

Modern injectable TCM formulations represent a key interface between traditional pharmacotherapy and modern intensive care practice. Xuebijing Injection, a compound containing extracts of Carthamus, Paeonia, Ligusticum, Salvia, and Angelica, has been extensively studied in sepsis management. Multiple RCTs and meta-analyses suggest that adjunctive administration of Xuebijing to standard sepsis care accelerates reductions in circulating TNF-α, IL-6, and PCT, improves coagulation parameters, shortens vasopressor duration, and may reduce 28-day mortality [173, 174]. Mechanistically, its effects involve anti-endotoxin, anti-inflammatory, immunomodulatory, and microcirculation-improving activities. Another example is Shenmai injection, derived from Panax ginseng and Ophiopogon japonicus. Traditionally indicated for supplementing Qi and nourishing Yin, Shenmai injection has shown potential benefits in septic shock, including improving hemodynamic stability and protecting myocardial function.

TCM interventions during this acute phase must be implemented strictly as adjunctive therapies and should not replace evidence-based standard-of-care measures, including early antibiotic administration, source control, and organ support. The optimal timing of administration (preferably early within the first 24 hours of diagnosis) and patient selection based on TCM pattern differentiation remain areas requiring active research.

3.3.3 Recovery and post-sepsis phase (immunosuppressive/prolonged inflammation stage): restoring homeostasis and functional recovery

After the acute hyperinflammatory phase, patients often face a prolonged period characterized by immune dysregulation, catabolic depletion, and functional decline. In TCM theory, this stage is commonly described as deficiency of both Qi and Yin (Qi-Yin Liangxu), accompanied by residual blood stasis and phlegm-damp accumulation. Therapeutic focus shifts to tonification, elimination of residual pathogenic factors, and promotion of tissue repair.

Formulas such as Shengmai San (composed of Ginseng, Ophiopogon, Schisandra) or Liuwei Dihuang Wan are commonly used to replenish vital energy and fluids that depleted by the hypercatabolic state of sepsis. Clinically, such approaches aim to ameliorate fatigue, anorexia, and generalized weakness. Atractylodes macrocephala (Bai Zhu) and Poria cocos (Fu Ling) are used to strengthen spleen function and resolve dampness, addressing anorexia and digestive weakness. Mild blood-activating herbs may be continued judiciously to facilitate recovery of organ function.

Emerging evidence suggests that several TCM herbs have prebiotic effects and can modulate gut microbial composition and associated gut–lung and gut–brain axes. Restoring intestinal barrier integrity and microbial homeostasis aligns with the TCM concept of fortifying the "acquired foundation" (Spleen-Stomach system), which is crucial for overall recovery and immune reconstitution. Adjunctive non-pharmacological modalities, such as acupuncture and moxibustion, can further support recovery. Acupuncture at points like Zusanli (ST-36), Guanyuan (CV-4), and Qihai (CV-6) can help regulate immune function, improve gastrointestinal motility, reduce residual inflammatory burden, and address symptoms like pain, insomnia, and cognitive dysfunction associated with post-sepsis syndrome.

In conclusion, perioperative sepsis is a time-sensitive, pathophysiologically complex condition requiring stage-specific management strategies. TCM, with its rich pharmacopeia and sophisticated diagnostic framework centered on pattern differentiation and temporal progression, offers valuable tools for modulating the host response across each stage. When applied as evidence-informed adjunctive therapy within a standardized perioperative care pathway, TCM interventions may contribute to improved immune regulation, microcirculatory stability, and functional recovery. Future research should focus on high-quality, multicenter RCTs incorporating standardized biomarker panels and outcome assessments to further refine this integrative temporal strategy and solidify its role in evidence-based sepsis management.

4 CONCLUSION

In recent years, accumulating evidence has elucidated the multi-dimensional mechanism by which TCM extracts alleviates sepsis-associated myocardial injury, demonstrating unique advantages beyond conventional single-target pharmacotherapies [175-177]. These natural compound preparations exert synergistic regulatory effects by concurrently modulating oxidative stress, suppressing excessive inflammation, maintaining mitochondrial function, and enhancing the anti-apoptotic ability of cardiomyocytes. More importantly, this holistic regulatory concept is highly consistent with the evolving trend in critical care medicine, which is shifting from a predominantly "pathogen-eradication–oriented" strategy to an "immune homeostasis reconstruction and organ protection" paradigm. Collectively, these findings suggest that TCM may serve not only as an auxiliary means but also as a source of novel theoretical frameworks and intervention paths for the treatment of sepsis.

However, there are still substantial translational bottlenecks. Most supportive data are derived from animal models, such as LPS or CLP models. Although these models are convenient to operate, they inadequately recapitulate the dynamic evolution of human sepsis - especially late-stage immune suppression, metabolic reprogramming, and interdependent multi-organ failure. The resulting "efficacy illusion" may lead to an overestimation of clinical value. Moreover, the inherent chemical complexity of TCM extracts—some formulations containing dozens of bioactive constituents—poses challenges in identifying definitive pharmacologically active components, characterizing pharmacokinetic profiles, and predicting drug–drug interactions (such as vancomycin, norepinephrine) in the intensive care setting. Without standardized extraction processes, rigorous quality control metrics, and reliable PK/PD models, even formulations with robust preclinical performance may face significant barriers to precise clinical application.

Safety considerations are equally critical. The assumption that "natural equates safe" should be avoided. Reports indicate that astragalus injection may be associated with immediate hypersensitivity reactions, the components of danzhenyuan may inhibit the CYP3A4 enzyme and thereby affect the metabolism of antibiotics, and long-term use of Xuebijing may occasionally lead to an increase in transaminase or creatinine levels in patients with liver and kidney dysfunction [178-180]. Although the overall incidence of such adverse events appears relatively low, even rare complications may have serious consequences in critically ill patients. Therefore, it is urgent to establish dedicated pharmacovigilance system for TCM injections, develop organ function–based individualized medication guidelines, and clearly delineate potential adverse reactions and contraindications in the product labeling.

Looking forward, advancing this field from empirical exploration to evidence-based integration requires breakthroughs in at least three key domains. First, high-quality multicenter RCTs should be designed with patient-centered outcomes, not only focusing on 28-day mortality rates, but also including comprehensive endpoints such as the speed of cardiac function recovery, length of ICU stay, and quality of life at 6 months; Second, an integrated research framework linking mechanistic studies, clinical trials and real-world data should be established. Emerging technologies such as organoids, single-cell sequencing, and artificial intelligence may facilitate systematic characterization of the multi-component, multi-target properties of TCM formulations; Third, interdisciplinary collaboration among experts in TCM, critical care medicine, pharmacology, toxicology, and regulatory science is essential to jointly formulate a research path for TCM compound preparations that conforms to international standards. Only in this way can the thousand-year wisdom of TCM be transformed into repeatable, scalable, and reliable modern critical care strategies, providing more effective and more humanistic care options for patients with sepsis worldwide.

DECLARATIONS

Author contributions

Yutong Sun, Qin Zhang, and Yan Zhang, contributed to the conception of the study and wrote the manuscript draft; Jiayin Wang, Weiqi Lin, Sixu Chen, Haiyi Qian, Xinyi Xie, Qixiang Xu and Xiaolong Yuan contributed to the information collection and analysis; Cuifeng Zhang helped perform the analysis by having constructive discussions and revising the manuscript.

Funding

This work was supported by Natural Science Foundation of the Higher Education Institutions of Anhui Province (2023AH051762, 2024AH051958), The Foundation of Wannan Medical College (WK2023ZQNZ08), National university innovation and entrepreneurship training program (202310368014, 202310368049, 202410368018, 202410368019, 202410368014),

Anhui Province university innovation and entrepreneurship training program (S202310368095, S202310368087, S202410368004, S202410368009).

Data availability

Not applicable.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

This work was supported by Lunan Houpu Pharmaceutical Co., Ltd. Horizontal Research Project (HXKT2022012).

Acknowledgements

Not applicable.

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Perioperative Precision Medicine

Zentime

RESEARCH ARTICLE

Elevated red cell distribution width opon ICU admission independently predicts mortality in young patients with sepsis-associated encephalopathy: A propensity score-matched retrospective cohort study using MIMIC-IV database

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Received November 18, 2025; Accepted March 3, 2026; Published March 31, 2026

DOI: 10.61189/402108pvrojs

Abstract

Background: Current prognostic research on sepsis-associated encephalopathy (SAE) predominantly focuses on elderly populations, while independent risk markers for young patients remain unclear. **Objective:** To investigate the prognostic value of red cell distribution width (RDW) for 30-day mortality in young SAE patients admitted to the intensive care unit (ICU). **Methods:** This retrospective cohort study analyzed 1,594 SAE patients (18-65 years) from the MIMIC-IV database. Using propensity score matching (1:1 nearest-neighbor matching), 352 matched pairs were generated. RDW-mortality association was assessed through restricted cubic splines, multivariable Cox regression, and Kaplan-Meier analysis. **Results:** Among the 1,594 young patients with SAE analyzed, 144 subjects (9.0%) passed away within 30 days following their ICU admission. Non-survivors exhibited significantly higher baseline RDW than survivors (17.5±3.1 versus 14.9±2.4, P<0.001). Patients exhibiting elevated RDW showed higher rates of hepatic disorders, clotting dysfunction, and impaired kidney function (all P<0.001). RDW and 30-day post-ICU admission mortality were nonlinearly related. After matching (standardized mean difference <0.1 for all covariates), higher RDW values showed a notable association with greater mortality risk over a 30-day period (hazard ratio [HR]=2.7, 95% confidence interval [CI]: 1.4-5.3, P=0.003). Also, after comprehensive adjustment for covariates, each 1-unit increase in RDW was still associated with a 20% rise in the risk of death (HR=1.2, 95% CI: 1.1-1.4, P<0.001). Kaplan-Meier curves confirmed reduced 30-day survival in high-RDW group (log-rank test, P=0.002). **Conclusions:** Elevated RDW at ICU admission independently predicts 30-day mortality in young SAE patients.

Keywords: Sepsis-associated encephalopathy, 30-day mortality in intensive care unit, Red cell distribution width, Predictor, Young patients, MIMIC-IV database

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Highlights

- Elevated red cell distribution width (RDW) (>14.65%) at intensive care unit admission independently predicts 30-day mortality in young patients with sepsis-associated encephalopathy (SAE) (hazard ratio =2.7, 95% confidence interval [CI]: 1.4-5.3; P=0.003), persisting after rigorous propensity score matching (352 pairs) and multivariable adjustment.
- RDW achieved an area under the curve of 0.760 (95% CI: 0.720-0.800), outperforming prior reports in elderly sepsis cohorts and underscoring its specificity for young SAE.
- RDW is a low-cost, routinely available biomarker. Its integration into risk stratification could enable early intervention in resource-limited settings, challenging the paradigm of youth conferring low risk in SAE.

1 INTRODUCTION

Sepsis-associated encephalopathy (SAE) represents a major medical complication. The occurrence rate ranges between 8% and over 70%, with variations based on how severe the sepsis condition is in patients requiring critical care [1]. The clinical manifestation of SAE is an acute disorder of consciousness with delirium, hallucinations and agitation [2]. Sepsis is often accompanied by inflammation and coagulation problems. Consequently, systemic inflammation induces SAE by activating endothelial cells and microglial cells, leading to increased blood-brain barrier penetrability and subsequent damage to brain tissue [1, 3, 4]. SAE has been demonstrated to be closely associated with increased short-term mortality and with impairment of cognitive function, anxiety and stress-related disorders; therefore, accurate and convenient assessment of outcomes is particularly crucial [2, 5]. For the clinical front line, a biomarker that is both simple and reliable and can be repeatedly measured at the bedside can help to identify high-risk patients early and guide subsequent treatment decisions. Most existing studies have focused on elderly patients with SAE, whereas younger patients, whose physiological reserve is greater and who are often regarded as a "low-risk population", are instead easily overlooked in research and in clinical alertness [6, 7]. In this population, once SAE occurs, the 30-day mortality rate can still be as high as 9% (data from the present study), and this figure in itself suggests that it is necessary to establish as soon as possible a dedicated early warning tool for young patients.

Red cell distribution width (RDW) is used to quantify the variation in red blood cell (RBC) volume; it reflects the balance between production and clearance of RBCs and can also serve as a simple indicator of acute and chronic systemic inflammatory burden [8]. When RBC homoeostasis is severely disrupted, RDW is usually increased, a phenomenon that is very common in patients with severe infection and can also be seen in various metabolic disorders such as inflammatory diseases, malnutrition and lipid metabolic disturbances [9-11]. An increasing number of studies have found that among populations at intensive care unit (ICU), RDW levels are closely related to patients' survival outcomes and can be used to assess the risk of death in various diseases such as sepsis, diabetes, and hepatic and renal failure [9, 10, 12-15]. It is noteworthy that RDW has also gradually shown potential to reflect the prognosis of encephalopathy and brain injury; the studies by Peng et al. and Yang et al. support the view that RDW can serve as a predictive factor for septic encephalopathy [16, 17]. Additionally, a recent study has proposed a correlation between heightened RDW levels in individuals with traumatic brain injury and increased mortality rates, as well as unfavorable neurological outcomes [18]. Kenangil et al. discovered that Parkinson's disease patients with neuroinflammation as a pathological feature had significantly higher RDW than healthy subjects [19].

While these initial findings propose a plausible association between RDW and brain disorders, further extensive investigations are warranted to authenticate and expand upon these preliminary observations. The predictive value of initial RDW measurements at ICU admission for SAE patient outcomes remains unclear. The goal is to find out how initial RDW relates to ICU prognosis in young patients with SAE, which could facilitate risk stratification.

2 METHODS

2.1 Data sources

The complete collection of patient health states and diseases was constructed utilizing information derived from the openly available Medical Information Mart for Intensive Care, version 4 (MIMIC-IV) database, which contains the Beth Israel Deaconess Medical Center's medical records from 2008 to 2019 [20, 21]. The MIMIC-IV database, a comprehensive healthcare repository featuring anonymized, high-quality clinical data, includes approximately 10,000 recorded ICU stays. The author, JH, was permitted to retrieve and analyze database information. The utilization of these data was endorsed by Massachusetts Institute of Technology and Beth Israel Deaconess Medical Center's Institutional Ethics Board, who also waived informed consent requirements (credential ID: 55826703).

2.2 Cohort selection

In this retrospective study, we examined SAE patients younger than 65 using MIMIC-IV database entries. SAE was defined by the occurrence of sepsis plus either reduced consciousness (Glasgow Coma Scale scores <15) or delirium symptoms. The

identification of sepsis followed Sepsis-3 criteria, requiring suspected infection with an increase in sequential organ failure assessment (SOFA) score to 2 or above. Patient selection was conducted according to the following eligibility standards: (1) first entry into the ICU; (2) length of ICU admission greater than 24 hours. Patients were not eligible if they met any of the following exclusion criteria: (1) primary cerebral trauma; (2) pre-existing mental health conditions or neurologic disorders; (3) development of metabolic, hepatic, or hypertensive encephalopathy, or any hepatic/renal conditions affecting mental status; (4) presence of severe electrolyte or glucose abnormalities, specifically sodium levels below 120 mmol/L, blood glucose above 180 mg/dL, or below 54 mg/dL; (5) documented delirium related to dementia, alcohol withdrawal, or medication use; (6) age ≥65 years; (7) missing RDW data. The process diagram is illustrated in Supplementary Figure 1.

2.3 Data exaction

The original data was obtained from the MIMIC-IV dataset through Structured Query Language executed using Navicat Premium 15.0.12. Data integration was performed using Stata MP 17. The gathered data included: (1) population-related and hospitalization statistics: patient's age, sex, racial background, hospital length of stay (LOS), and LOS in ICU; (2) physical signs and measurements: heart rate, systolic blood pressure (SBP), diastolic blood pressure (DBP), mean blood pressure (MBP), respiratory rate, and temperature; (3) existing health conditions: myocardial infarction, congestive heart failure, chronic pulmonary disease, renal disease, liver disease, diabetes, and peripheral vascular disease; (4) patient assessment metrics: SOFA score, logistic organ dysfunction system score , and simplified acute physiology score II; (5) treatment procedures: use of vasoactive drugs and invasive mechanical ventilation; (6) laboratory tests: white blood cell count, hemoglobin concentration, mean corpuscular volume, platelet count, RBC count, anion gap, bicarbonate levels, electrolyte levels including sodium, potassium, calcium, and chloride, renal function indices including blood urea nitrogen (BUN) and creatinine, glucose, and blood clotting markers including international normalized ratio (INR), prothrombin time (PT), activated partial thromboplastin time (APTT), and RDW [20-22]. It was within the first 24 hours post-ICU admission that these baseline demographic data and assay findings were obtained. For parameters with multiple measurements within this initial 24-hour window, average values were computed, specifically for heart rate, SBP, DBP, MBP, respiratory rate, and body temperature.

2.4 Outcomes

In our research, the main outcome measure was mortality within 30 days following ICU admission. Secondary endpoints encompassed overall in-hospital death rate, duration of hospitalization, and time spent in intensive care.

2.5 Management of missing data

To reduce potential bias, we excluded from our examination any variables containing over 15% missing values. The small number of remaining missing data points was imputed using mean values.

2.6 Statistical analysis

According to the distribution characteristics of the data, continuous variables were expressed as mean ± standard deviation or median (interquartile range), and comparisons between groups were performed using the Mann–Whitney test or Student's t test; categorical variables were expressed as percentages, and Fisher's exact test was used to assess differences. We plotted receiver operating characteristic (ROC) curves to evaluate the ability of RDW to predict 30-day mortality risk in the included patients, and, by means of a generalized additive model with restricted cubic splines, explored whether there was a non-linear association between continuous RDW and the primary outcome. The optimal cut-off value of RDW was determined on the basis of the ROC curve, after first including RDW as a continuous variable in the analysis; subsequently, survival curves were plotted by the Kaplan–Meier method, and survival differences between groups were compared using the log-rank test.

To minimize selection bias and balance baseline characteristics as far as possible, we conducted a one-to-one nearest-neighbour propensity score matching (PSM) analysis with a caliper width of 0.02. Each patient's propensity score was calculated by logistic regression, in which the baseline variables listed in **Table 1** were included. After completing PSM, we compared the standardized mean differences between the two groups, taking 0.15 as the upper limit for acceptable balance.

In order to further evaluate the relationship between RDW and clinical outcomes, we constructed univariable and multivariable Cox regression models respectively. Variables with a P value less than 0.1 in the univariable analysis after PSM were included in the multivariable model to control potential confounders. After matching was completed, we tested the proportional hazards assumption of the Cox model to confirm that there were no time-varying covariates in the adjusted model. All statistical analyses were performed in the R 4.2.0 environment, and a difference was considered statistically significant when $P<0.05$.

3 RESULTS

3.1 Clinical characteristics

A total of 1,594 subjects were included in this study, with a mean age of 49.8±12.1 years, of whom 63.0% were male. Within 30 days after ICU admission, a total of 144 patients (9.0%)

Table 1. Clinical characteristics stratified by preoperative RDW before and after PSM

Variables	Unmatched groups			Matched groups		
	RDW≤14.65 (n=859)	RDW>14.65 (n=735)	P-value	RDW≤14.65 (n=352)	RDW>14.65 (n=352)	P-value
Demographics						
Age, y	47.8±13.1	52.2±10.2	<0.001	51.0±12.2	51.5±10.7	0.597
Male	595 (69.3%)	410 (55.8%)	<0.001	205 (58.2%)	216 (61.4%)	0.398
Race			0.007			0.754
White	485 (56.5%)	428 (58.2%)		211 (59.9%)	202 (57.4%)	
Black	61 (7.1%)	78 (10.6%)		35 (9.9%)	41 (11.6%)	
Others	96 (11.2%)	87 (11.8%)		36 (10.2%)	42 (11.9%)	
Unknown	217 (25.3%)	142 (19.3%)		70 (19.9%)	67 (19.0%)	
Comorbidities						
Myocardial infarction	76 (8.8%)	58 (7.9%)	0.493	32 (9.1%)	33 (9.4%)	0.896
Congestive heart failure	130 (15.1%)	138 (18.8%)	0.053	70 (19.9%)	68 (19.3%)	0.849
Chronic pulmonary disease	160 (18.6%)	195 (26.5%)	<0.001	90 (25.6%)	82 (23.3%)	0.483
Renal disease	59 (6.9%)	107 (14.6%)	<0.001	41 (11.6%)	39 (11.1%)	0.812
Liver disease	133 (15.5%)	342 (46.5%)	<0.001	95 (27.0%)	93 (26.4%)	0.865
Diabetes	109 (12.7%)	128 (17.4%)	0.008	52 (14.8%)	53 (15.1%)	0.916
Peripheral vascular disease	61 (7.1%)	41 (5.6%)	0.216	25 (7.1%)	26 (7.4%)	0.884
Vital signs						
HR, (beats/min)	90.4±17.3	92.6±16.6	0.009	91.8±17.7	92.2±17.0	0.765
SBP, mmHg	115.5±14.8	112.6±14.5	<0.001	114.0±14.7	112.9±14.2	0.318
DBP, mmHg	66.4±10.5	64.0±10.0	<0.001	66.1±9.9	65.1±10.2	0.217
MBP, mmHg	80.0±10.5	77.3±10.4	<0.001	79.4±9.8	78.4±10.5	0.185
RR, (breaths/min)	19.9±4.3	20.0±4.7	0.517	20.2±4.3	20.1±4.7	0.664
Temperature, ℃	37.2±0.6	37.1±0.5	<0.001	37.1±0.6	37.2±0.5	0.678
SpO_2, %	97.2±2.2	96.9±2.1	0.013	97.1±2.1	97.1±2.0	0.864
Scores						
SOFA	6.6±3.5	9.0±4.3	<0.001	7.5±3.6	7.7±3.9	0.457
LODS	5.7±3.0	7.2±3.5	<0.001	6.2±3.2	6.4±3.1	0.474
SAPS II	33.1±11.9	39.6±13.8	<0.001	35.4±12.8	36.3±11.8	0.326
Treatment						
Vasoactive agent	432 (50.3%)	412 (56.1%)	0.022	188 (53.4%)	192 (54.5%)	0.762
Invasive Ventilation	616 (71.7%)	488 (66.4%)	0.022	243 (69.0%)	242 (68.8%)	0.935
Laboratory parameters						
WBC, 10^9/L	13.0±6.4	12.9±11.6	0.004	12.6±7.1	12.8±8.0	0.695
Hemoglobin, g/dl	11.8±2.2	9.7±2.2	<0.001	10.8±2.2	10.6±2.3	0.188
MCV, fl	92.6±6.2	92.5±10.1	0.788	92.7±7.0	93.0±8.8	0.637
PLT, 10^9/L	194.1±85.6	168.4±125.4	<0.001	183.6±93.6	189.2±131.7	0.512
RBC, 10^{12}/L	3.8±0.7	3.3±0.8	<0.001	3.6±0.8	3.5±0.8	0.190
Anion gap, mmol/L	14.8±4.3	15.8±4.9	<0.001	15.0±5.0	15.1±4.7	0.825
Bicarbonate, mmol/L	22.5±4.6	21.7±5.1	<0.001	22.5±5.1	22.2±4.9	0.369
BUN, mg/dl	20.1±19.4	31.5±29.3	<0.001	24.1±25.5	25.1±22.2	0.584
Calcium, mg/dl	8.1±0.8	8.1±1.0	0.502	8.0±0.9	8.0±0.9	0.618
Chloride, mmol/L	104.7±6.5	103.5±7.6	0.001	104.2±7.0	104.6±7.3	0.395
Creatinine, mg/dl	1.3±1.6	1.8±2.0	<0.001	1.4±1.7	1.5±1.8	0.564
Glucose, mg/dl	122.8±25.7	119.6±27.4	0.015	122.4±25.9	120.6±27.3	0.371
Sodium, mmol/L	138.9±4.8	138.1±5.7	0.001	138.7±5.2	138.9±5.5	0.511

Potassium, mmol/L	4.1±0.7	4.2±0.9	0.109	4.1±0.7	4.1±0.8	0.918
INR	1.4±0.5	1.7±0.8	<0.001	1.5±0.7	1.5±0.6	0.613
PT, s	15.0±5.6	18.6±11.3	<0.001	16.2±7.2	16.5±6.6	0.605
APTT, s	34.9±18.8	40.3±22.5	<0.001	37.3±22.2	38.1±20.9	0.614

Note: Values are expressed as number (percentage), mean ± standard deviation, or median (25^{th}-75^{th} percentile). Categorical variables are presented as number (percentage). HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; RR, respiratory rate; SOFA, sequential organ failure assessment; LODS, logistic organ dysfunction system; SAPS II, simplified acute physiology score II; WBC, white blood cells; MCV, mean corpuscular volume; PLT, platelet; RBC, red blood cells; BUN, blood urea nitrogen; INR, international normalized ratio; PT, prothrombin time; APTT, activated partial thromboplastin time; RDW, red blood cell distribution width; PSM, propensity score matching.

Table 2. Clinical outcomes in the SAE patients

Variables	Unmatched groups			Matched groups		
	RDW≤14.65 (n=859)	RDW>14.65 (n=735)	P-value	RDW≤14.65 (n=352)	RDW>14.65 (n=352)	P-value
30-day in-ICU mortality	26 (3.0%)	118 (16.1%)	<0.001	12 (3.4%)	35 (9.9%)	<0.001
Hospital mortality, day	13 (1.5%)	81 (11.0%)	<0.001	6 (1.7%)	24 (6.8%)	<0.001
LOS ICU, day	7.5±7.6	7.6±7.5	0.673	7.5±7.2	7.3±6.6	0.742
LOS hospital, day	17.2±17.7	20.3±17.7	<0.001	17.8±16.5	18.5±14.4	0.558

Note: Values are expressed as mean ± standard deviation or number (percentage). SAE, sepsis-associated encephalopathy; RDW, red blood cell distribution width; ICU, intensive care unit; LOS, length of stay.

died, indicating that even in a relatively young population, short-term mortality risk cannot be ignored. As shown in Supplementary Figure 2, there were obvious differences in RDW levels between groups, and the RDW of patients who died was significantly higher than that of survivors (17.5±3.1 vs 14.9±2.4, P<0.001).

Based on ROC curve analysis, the optimal RDW cut-off value was 14.65, with a corresponding area under the curve (AUC) of 0.760 (95% confidence interval [CI]: 0.720-0.800) (Supplementary Figure 3), according to which the study population was divided into a low-RDW group (n=859) and a high-RDW group (n=735). Comparison of the clinical characteristics of young SAE patients (**Table 1**) showed that, compared with the low-RDW group, patients in the high-RDW group were older, had comorbidities more frequently (except for myocardial infarction and peripheral vascular disease), had higher heart rates and higher disease severity scores, while their blood pressure, body temperature and SpO_2 were relatively lower. Furthermore, the elevated RDW category showed higher anion gap, BUN, creatinine, INR, PT, and APTT values (all P<0.05). In contrast, mean corpuscular volume, calcium, and potassium showed no significant difference between groups, while other parameters were significantly lower in the high-RDW group (all P<0.05).

3.2 PSM procedure

We conducted a 1:1 PSM analysis to equalize covariate distribution between subjects categorized by RDW thresholds, resulting in 352 matched pairs. **Table 1** presents the clinical features of young SAE patients post-matching. Both groups showed comparable distributions of demographics, comorbidities, signs, scores, and laboratory values. The matching quality was evaluated through standardized mean difference calculations pre- and post-PSM (Supplementary Figure 4).

3.3 RDW levels and primary outcomes

The mortality rate at 30 days showed a significant elevation among matched subjects with elevated RDW values (9.9% compared to 3.4%, P<0.001) (**Table 2**). Increased RDW demonstrates an independent correlation with elevated 30-day mortality among young ICU patients experiencing SAE (Supplementary Tables 1 and 2).

Through multivariable Cox proportional hazards analysis evaluating RDW as a continuous parameter, this biomarker demonstrated significant predictive capacity for 30-day ICU mortality in the matched cohort (HR, 1.3 [95% CI, 1.2-1.4], P<0.001). In comparison to individuals with low RDW levels (≤14.65), young ICU patients diagnosed with SAE demonstrated an increased hazard ratio (95% CI) of 2.7 (1.4-5.3) for overall mortality. Other parameters emerged as independent predictors including patient age, liver disease, and body temperature (Supplementary Tables 1 and 2). As a continuous variable, RDW maintained status as an independent predictor of elevated hospital mortality risk (HR, 1.2 [95% CI, 1.1-1.4], P<0.001) (**Table 3**). Following sequential adjustments for demographic factors, comorbid conditions, vital signs, clinical scores, therapeutic measures and pharmacological treatments, and laboratory indicators, the observed correlations in matched patients

Table 3. Association of RDW with outcomes after PSM

Clinical outcome	Unadjusted	Model I	Model II	Model III	Model IV	Model V
30-day in-ICU mortality						
RDW continuous	1.3 (1.2, 1.4) <0.001	1.3 (1.2, 1.4) <0.001	1.2 (1.1, 1.4) <0.001	1.2 (1.1, 1.4) <0.001	1.2 (1.1, 1.4) <0.001	1.2 (1.1, 1.4) <0.001
RDW categorical						
≤14.65	1	1	1	1	1	1
>14.65	2.7 (1.4, 5.3) 0.003	2.8 (1.4, 5.4) 0.002	3.0 (1.6, 5.9) 0.001	2.8 (1.4, 5.8) 0.005	3.0 (1.4, 6.2) 0.004	3.4 (1.5, 7.5) 0.003
Hospital mortality						
RDW continuous	1.2 (1.1, 1.4) <0.001	1.2 (1.1, 1.4) <0.001	1.2 (1.1, 1.4) 0.001	1.2 (1.0, 1.4) 0.016	1.2 (1.0, 1.4) 0.018	1.2 (1.0, 1.4) 0.099
RDW categorical						
≤14.65	1	1	1	1	1	1
>14.65	3.7 (1.5, 9.2) 0.004	4.0 (1.6, 9.8) 0.003	4.0 (1.6, 9.8) 0.003	3.5 (1.3, 9.6) 0.017	3.3 (1.2, 9.5) 0.023	3.2 (1.0, 10.1) 0.052

Note: Values are expressed as HR (95% CI). Model I adjusted for demographics including gender, age, race. Model II adjusted for adjust I model plus comorbidities including myocardial infarction, congestive heart failure, chronic pulmonary disease, renal disease, liver disease, diabetes, peripheral vascular disease. Model III adjusted for adjust II model plus vital signs and scores including HR, SBP, DBP, MBP, RR, temperature, SpO_2, SOFA, LODS, and SAPS II. Model IV adjusted for adjust III model plus treatment including vasoactive agent, and invasive ventilation. Model V adjusted for adjust IV model plus laboratory parameters including WBC, hemoglobin, MCV, PLT, RBC, anion gap, bicarbonate, BUN, calcium, chloride, creatinine, glucose, sodium, potassium, INR, PT, and APTT. HR, hazard ratio; CI, confidence interval; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; RR, respiratory rate; SOFA, sequential organ failure assessment; LODS, logistic organ dysfunction system; SAPS II, simplified acute physiology score II; WBC, white blood cells; MCV, mean corpuscular volume; PLT, platelet; RBC, red blood cells; BUN, blood urea nitrogen; INR, international normalized ratio; PT, prothrombin time; APTT, activated partial thromboplastin time; RDW, red blood cell distribution width; PSM, propensity score matching; ICU, intensive care unit.

maintained statistical significance, as demonstrated in **Table 3**. Nevertheless, upon implementing adjustments in Model V, the association between RDW and hospital mortality risk lost statistical significance (all P>0.05).

Survival analysis indicated significantly poorer 30-day ICU survival in young SAE patients with RDW>14.64% (log-rank test, P=0.002; **Figure 1**).

It was through restricted cubic spline modeling that the nonlinear correlation between RDW and 30-day ICU mortality was shown, where incremental RDW elevations predicted worsening survival probabilities (Likelihood ratio test, P<0.001) (**Figure 2**).

4 DISCUSSION

This study shows that among young patients with SAE, an elevated RDW level (>14.65%) is an independent predictor of 30-day ICU mortality risk, and this association remains stable even after full adjustment for multiple covariates. It is worth emphasizing that we focused on a group that is often overlooked yet very crucial, namely young SAE patients aged 18-65 years with relatively good physiological reserve, whereas previous studies on SAE prognosis and RDW have mostly been based on elderly or mixed-age cohorts. Young patients usually have fewer comorbidities and stronger organ reserve, and the driving factors of their adverse outcomes may be essentially different from those in elderly populations; even so, this study still observed a 30-day mortality rate close to 9%, and RDW was consistently a robust independent predictor, a result that strongly challenges the ingrained notion that "being young equals low risk". At the same time, in young patients, an elevated RDW is more likely to reflect the acute blow to RBC homoeostasis caused by the current acute infectious event such as an intense inflammatory storm or microcirculatory dysfunction, rather than being driven mainly by long-term chronic diseases or confounding factors such as malnutrition; this relationship still exists after adjustment for multidimensional covariates, including comorbidities. Consequently, the RDW threshold (14.65%) and its predictive efficacy (AUC=0.760) identified in this study may be more specific to young SAE patients, and its clinical early-warning value in this population may have been underestimated in previous research.

Previously, studies documented RDW's role in detecting anemia [22, 23]. More recent investigations have demonstrated RDW's connections to numerous infectious and inflammatory conditions [24-27]. In individuals diagnosed with COVID-19, RDW levels were significantly higher among those who developed severe disease or died, as opposed to those who exhibited mild symptoms or recovered from the infection [28-30]. Yeşil et al. revealed that patients with inflammatory bowel disease in the active phase showed higher RDW levels than those in

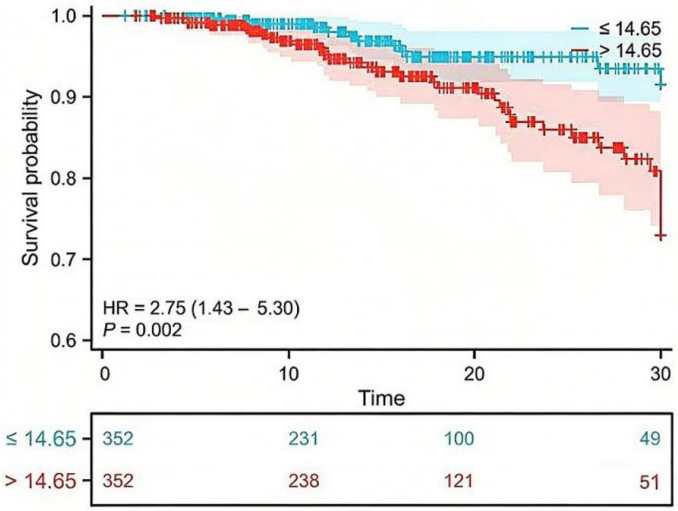

Figure 1. The KaplanMeier curves of 30-day outcomes after PSM. PSM, propensity score matching; HR, hazard ratio.

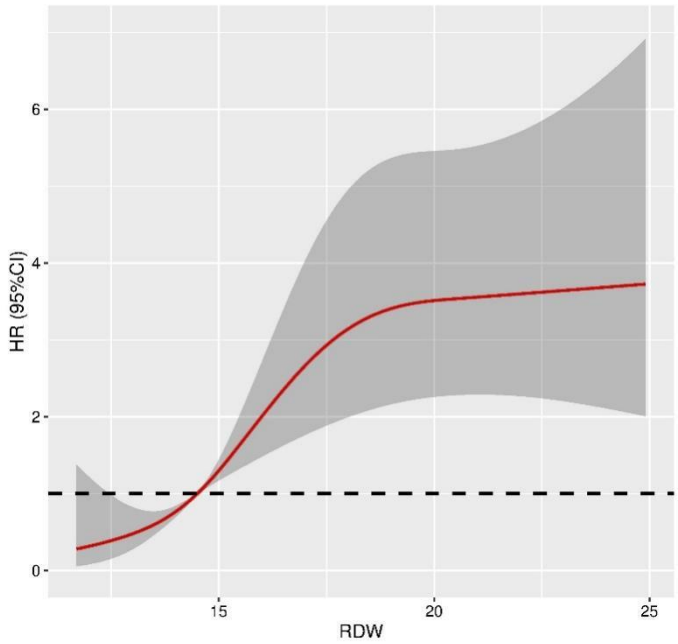

Figure 2. The association between RDW and the probability of 30-day outcomes. The red line represents the HR for each RDW value, while the gray bars represent the 95% confidence interval. RDW, red blood cell distribution width; HR, hazard ratio.

remission, indicating its possible role as a precise and responsive biomarker for identifying active Crohn's disease [24]. Additionally, findings from a retrospective analysis indicated that elevated RDW correlated with increased hospitalization rates and higher 30-day mortality among younger patients suffering from community-acquired pneumonia [25]. To our knowledge, this research represents the first investigation exploring RDW's correlation with short-term clinical outcomes in young SAE patients through analysis of population-level real-world evidence.

Alterations in blood coagulation mechanisms could account for the RDW-prognosis correlation among younger individuals with SAE. During septic conditions, substantial disruptions in coagulation processes occur. Under normal physiological conditions, the activation of blood clotting is regulated by intrinsic anticoagulation systems, differing from normal homeostasis maintained through tissue factor inhibition, protein C activation, and antithrombotic regulation [3]. Sepsis interferes with all three coagulation regulatory mechanisms. Research demonstrated that RDW showed positive associations with various prognostic indicators in multiple hepatic disorders, including serum bilirubin and creatinine concentrations, as well as PT measurements [31]. Elevated RDW is strongly associated with venous thrombosis and early death from acute pulmonary embolism [26, 32]. It is also possible for renal insufficiency to affect RBC production and platelet dysfunction [33, 34]. **Table 1** data revealed a correlation between rising RDW levels and increased incidence of liver-related conditions in this study, which was associated with elevated values of INR, PT, APTT, as well as increased BUN and creatinine levels. This, to some extent, reflects coagulation disorder, hepatic and renal dysfunction in these patients. Coagulation disorders in young SAE patients may be one of the potential mechanisms by which high level of RDW leads to poor outcomes.

An elevated RDW may indicate ineffective erythropoiesis or delayed clearance of erythrocytes, which may be influenced by disease, as it is an early indication of physiological stress [30, 35, 36]. Anisocytosis may indicate the presence of an underlying inflammatory condition, with cytokine release potentially influencing bone marrow functionality [37]. The RDW showed a robust and autonomous positive correlation with traditional markers of inflammation [38]. Inflammatory biomarkers have been used to diagnose, treat, and prognosticate sepsis patients [39, 40]. In septic encephalopathy, infection is accompanied by inflammation and stress. Reduced blood pressure and an elevated heart rate indicate the cardiac stress condition in individuals with high RDW, while the increased heart rate serves as a compensatory mechanism in response to sympathetic nervous system activation. Additionally, patients with high RDW had more extreme differences in white blood cell count.

Previous studies have already suggested that RDW is closely related to systemic inflammation and the prognosis of sepsis, and on this basis the present study approached the topic from a new angle, focusing on the early warning needs of young SAE populations. Most previous work on RDW and sepsis has included elderly or all-age cohorts, whereas young patients, who have stronger physiological reserve and are often labelled as low risk, are often neglected; however, we found that in this population the mortality rate still reached as high as 9%, and

the predictive performance of RDW (AUC=0.760) even exceeded previously reported values in elderly sepsis cohorts (AUC=0.710) [6, 7, 41]. This means that, in young SAE patients, RDW may capture acute injury signals that are more difficult for traditional risk models to identify, such as a more intense coagulation–inflammation cascade manifested as elevated INR, prolonged PT and impaired hepatic and renal function or microcirculatory dysfunction, rather than merely chronic organ failure, which is more common in older populations. At the same time, the independent value of RDW observed in this study challenges the traditional view that RDW is only a surrogate marker of inflammation. Even after strict PSM and comprehensive adjustment for covariates, RDW still maintained a significant predictive role (HR=1.2, P<0.001; **Table 3**), suggesting that the information it integrates also includes microcirculatory damage caused by abnormal RBC morphology, among which anisocytosis, that is, abnormal variation in RBC size, may disturb passage through cerebral capillaries and aggravate SAE-related ischemic and hypoxic injury.

On this basis, we further propose a more exploratory hypothesis: an elevated RDW may not only be a bystander of disease severity, but may also directly participate, through specific pathways, in neurological injury and adverse outcomes in SAE. Anisocytosis reduces the deformability of RBCs and their ability to pass through microvessels, particularly affecting cerebral microcirculation; against the background of sepsis, in which there is already uneven perfusion, stagnant blood flow and tissue hypoxia, an elevated RDW may further limit the delivery of oxygen and nutrients, thereby aggravating ischemic and hypoxic brain injury. The oxygen-carrying and oxygen-releasing functions of morphologically abnormal RBCs may also be impaired, and, in the context of increased metabolic demands of brain tissue during SAE, such functional defects may contribute to relative tissue hypoxia and drive the continuous progression of neurological dysfunction. It is also noteworthy that there is a bidirectional interaction between neuroinflammation in the brain and systemic inflammation and the RBC system: the core mechanisms of SAE include activation of microglia and the release of pro-inflammatory cytokines such as IL-6 and TNF-α, which can inhibit bone marrow hematopoiesis or shorten RBC lifespan; conversely, the intense systemic inflammation and potential oxidative stress reflected by elevated RDW may in turn, by disrupting the blood–brain barrier or through humoral signaling pathways, aggravate neuroinflammation within the brain and form a self-reinforcing vicious cycle [42, 43]. The characteristics and significance of this central–peripheral interaction in young patients with SAE still need to be elucidated systematically.

Compared with many complex or expensive tests, measurement of RDW is simple, rapid and easy to repeat; several studies have linked it with inflammatory diseases and thrombosis, suggesting that RDW may be closely related to the occurrence and prognosis of SAE [24-27]. Because it is affected by the lifespan of RBCs of about 3-4 months, RDW can integrate disease progression information over a relatively long time scale; this characteristic gives it a certain irreplaceability and also makes it promising in improving the accuracy of existing prognostic models for sepsis and SAE. The present study adopted a strict one-to-one PSM design, effectively balancing baseline differences, so that the significant association observed in the matched cohort between elevated RDW and 30-day mortality (HR=2.7, 95% CI: 1.4-5.3, P=0.003), as well as the obvious differences in survival rate between the high- and low-RDW groups (9.9% vs 3.4%, P<0.001), can more reasonably be attributed to RDW itself or to the pathophysiological states that it represents but have not yet been fully quantified, rather than to confounding factors. Relying on the large-scale, high-quality real-world database MIMIC-IV, the results of this study appropriately reflect the predictive performance of RDW in actual clinical settings, suggesting that this easily obtainable, low-cost routine test index has high potential for clinical translation, especially in settings with limited resources or without advanced bedside monitoring methods, where it can be used for rapid risk stratification and optimization of resource allocation.

Although in young SAE cases we observed a robust association between RDW levels and outcomes, this study still inevitably has limitations. The retrospective design based on the MIMIC-IV database may have introduced selection bias; even though PSM was used to reduce baseline differences as far as possible, residual confounding is still difficult to eliminate completely. Some variables in the database were missing, and even though appropriate handling has been carried out, their potential impact on the results still cannot be completely ruled out; at the same time, this study relied on a single database and lacked external cohort validation, so that the generalizability and applicability of the conclusions still need further testing. In addition, this study only evaluated the relationship between RDW and 30-day mortality, and the impact on more long-term outcomes such as long-term survival and quality of life has not yet been systematically assessed. The enrollment of SAE patients in our center is inherently challenging, primarily due to the limited number of eligible cases encountered in routine clinical practice. Therefore, we chose to use a public database for our initial SAE research.

Future research can build on the findings and limitations of this study and continue to expand. On the one hand, it is necessary to carry out large-scale prospective cohort studies covering multiple regions and multiple medical centers, in order to externally validate the results of this study and to compare the predictive performance of RDW for outcomes in young SAE patients under different clinical environments and treatment strategies; on the other hand, the intrinsic links between RDW, coagulopathy and inflammatory responses should be explored in depth, to clarify the specific biological pathways by which high RDW affects SAE prognosis and to provide clues for

potential therapeutic targets. Combining RDW with other novel biomarkers and clinical indicators is expected to construct a more comprehensive prognostic evaluation model for SAE and improve predictive accuracy; for young SAE patients with elevated RDW, strengthening follow-up and dynamic monitoring, particularly the assessment of cognitive function, mental health and overall quality of life, will also help to optimize clinical care and rehabilitation strategies.

On the basis of the new perspective proposed in this study, subsequent work can also focus on several directions with greater application prospects. At the clinical level, multicenter external validation studies are needed to systematically assess the generalizability, sensitivity and specificity of the RDW cut-off value (14.65%) determined in this study in independent and diverse cohorts of young SAE, and to further explore whether this threshold needs to be modified according to the source of infection such as Gram-positive or Gram-negative bacteria or fungi, pathogen virulence or the host genetic background. At the same time, the value of dynamic RDW trajectories should be a focus, for example changes at 24 h, 48 h, 72 h and even later time points after ICU admission, in order to observe whether they have earlier and more precise predictive ability than a single measurement and whether they can indicate disease progression trends, treatment responses such as the effects of anti-infective or anti-inflammatory therapies and the potential for neurological recovery. Another important goal is to construct integrated predictive models that combine RDW especially dynamic RDW with existing organ function scores such as SOFA and APACHE II, novel biomarkers such as the aforementioned markers of neuronal injury and specific inflammatory cytokines and feasible bedside microcirculatory or physiological function assessments, so as to promote the real implementation of precision medicine in critically ill young patients. To verify whether intensified treatment or refined monitoring strategies based on RDW risk stratification can improve short-term survival, neurological recovery and long-term outcomes, rigorously designed prospective randomized controlled trials are still needed, with comprehensive assessment of their risk–benefit ratio, cost-effectiveness and impact on medical resource allocation; this will be one of the key steps in elevating RDW from a simple prognostic marker to a tool for guiding treatment decisions.

5 CONCLUSION

Taken together, using a strict PSM method and large-scale real-world data, this study clearly delineated the level of short-term mortality in young patients with SAE and confirmed that elevated RDW at ICU admission is independently associated with 30-day mortality risk in this population. In doing so, it not only undermines the ingrained perception that young patients are naturally at lower risk, but also highlights the unique value of RDW in prognostic assessment among young SAE patients. Young SAE patients with high RDW levels should therefore receive special attention and more active management in clinical practice.

DECLARATIONS

Author contributions

Yalin Zhu, Zhengyu Jiang and Wangzheqi Zhang wrote the manuscript and performed the data analysis. Jie Huang, Haoling Zhang, Haiwen Wang and Wen Xu checked the data and the results. Yalin Zhu provided the funding of this study. Jiafeng Wang designed the study. Jiafeng Wang and Wen Xu revised the manuscript and supervised research. All authors have reviewed and approved the final version.

Funding

This study is supported by Zhoushan Medical and Health Technology Program (2023JYB01).

Data availability

The complete dataset referenced throughout our publications is accessible via the public MIMIC-IV database at https://mimic.mit.edu/iv/.

Ethics approval and consent to participate

This investigation adhered to the ethical standards of the Helsinki Declaration in 1964 and its subsequent amendments, or equivalent ethical guidelines, utilizing de-identified data obtained from the publicly accessible MIMIC-IV database. Access authorization was secured through certification ID 55826703 by researcher JH. Ethics approval was granted under a consent waiver protocol by the IRBs of Massachusetts Institute of Technology and Beth Israel Deaconess Medical Center.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

Not applicable.

Supplementary Information

The online version (of this article) contains supplementary material available at https://doi.org/10.61189/402108pvrojs.

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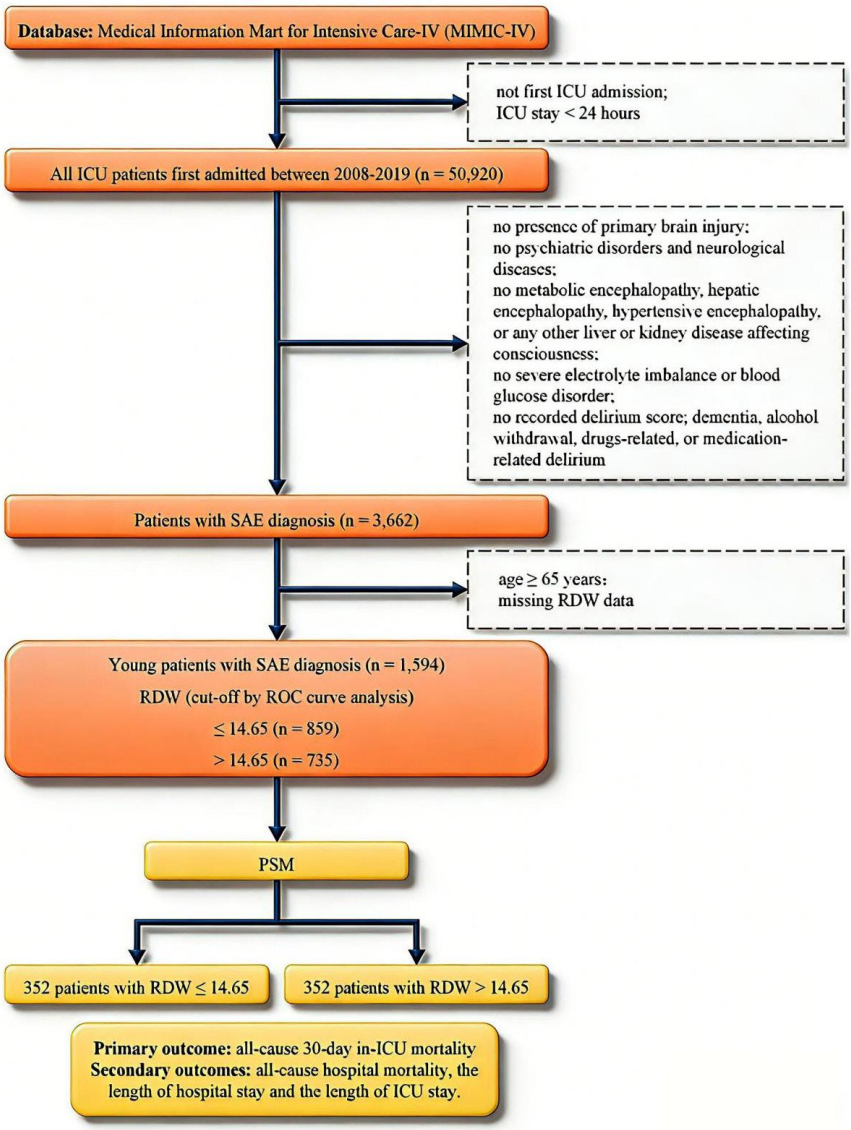
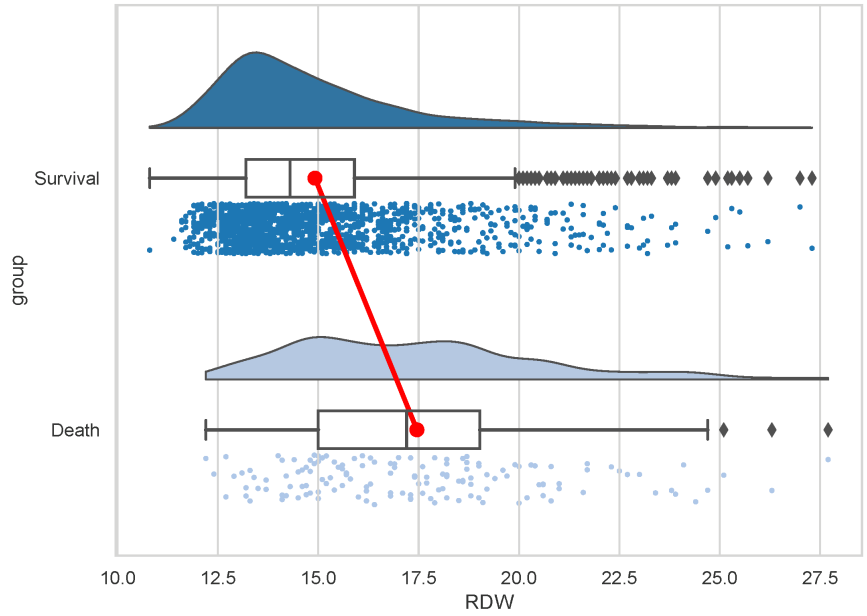
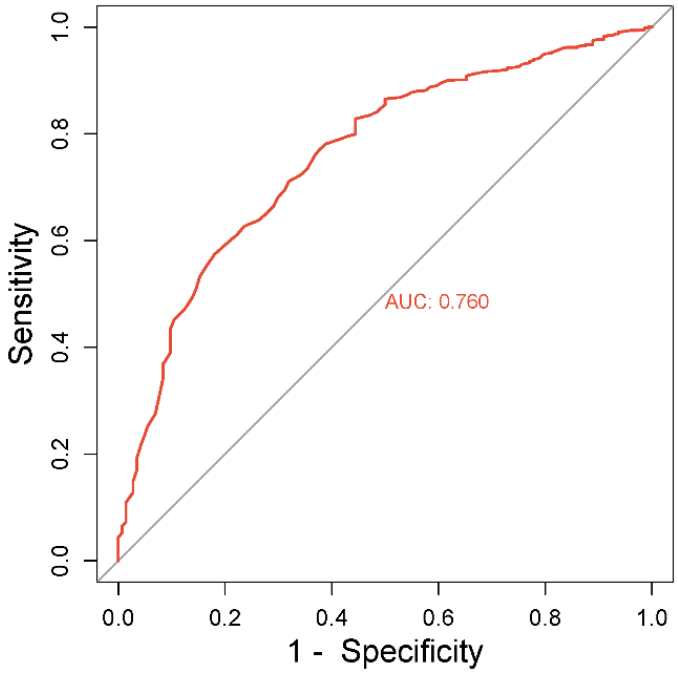
Supplementary Information



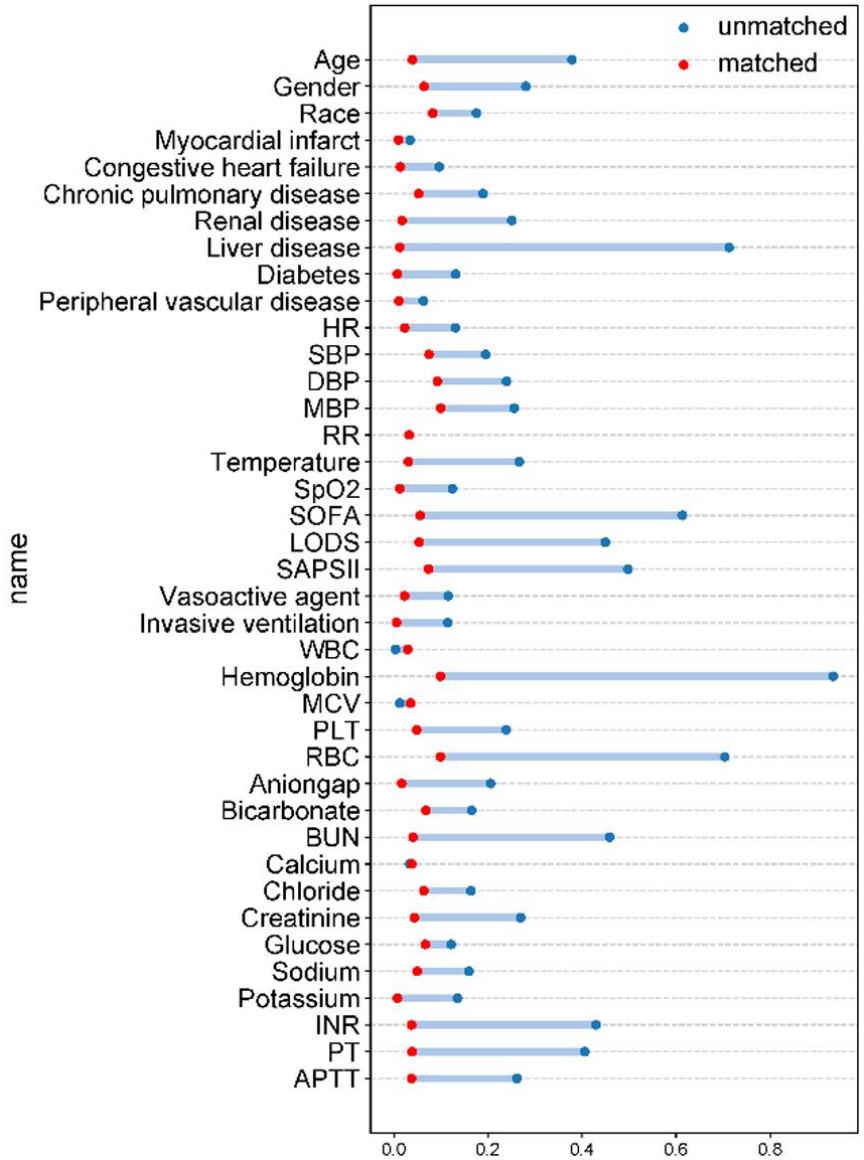
Supplementary Figure 1. Study flow chart. SAE, sepsis-associated encephalopathy; RDW, red blood cell distribution width; ICU, intensive care unit; ROC, receiver operating characteristic; PSM, propensity score matching.



Supplementary Figure 2. The different RDW levels in young SAE patients with different 30-day outcomes. The box plot represents the general distribution of the data. The boxes contain a red spot in the middle (the mean value of the data) and a line (the median). The jittered raw data (the 'rain') below the boxes is the RDW value of each case, and the 'cloud' above the boxes represents the number of young SAE patients with different RDW values. P<0.001. The differences were assessed with the Kruskal−Wallis test. SAE, sepsis-associated encephalopathy; RDW, red blood cell distribution width.



Supplementary Figure 3. The receiver operating characteristic curve for RDW in predicting all-cause 30-day death in the ICU. The cutoff value of RDW is 14.65%. AUC=0.760, 95% CI 0.720 to 0.800. ICU, intensive care unit; CI, confidence interval; AUC, area under curve; RDW, red blood cell distribution width.



Supplementary Figure 4. The SMD for variables of two groups before and after PSM. SMD, standard mean difference; PSM, propensity score matching; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; RR, respiratory rate; SOFA, sequential organ failure assessment; LODS, logistic organ dysfunction system; SAPS II, simplified acute physiology score II; WBC, white blood cells; MCV, mean corpuscular volume; PLT, platelet; RBC, red blood cells; BUN, blood urea nitrogen; INR, international normalized ratio; PT, prothrombin time; APTT, activated partial thromboplastin time.

Supplementary Table 1. Univariate and multivariate Cox regression model used to study the association of RDW with 30-day mortality in matched young SAE patients in ICU

	Univariate analysis			Multivariate analysis		
	HR	95% CI	P-value	HR	95% CI	P-value
Age, y	1.0	(1.0, 1.1)	0.007	1.1	(1.0, 1.1)	0.003
Female	0.8	(0.4, 1.5)	0.504			
Race						
White	Ref			Ref		
Black	1.1	(0.4, 2.8)	0.850	0.9	(0.3, 3.4)	0.915
Others	0.2	(0.0, 1.2)	0.071	0.1	(0.0, 1.2)	0.075
Unknown	1.2	(0.6, 2.5)	0.536	2.2	(0.9, 5.1)	0.066
Myocardial infarct	0.4	(0.1, 1.5)	0.151			
Congestive heart failure	0.6	(0.3, 1.4)	0.238			
Chronic pulmonary disease	1.8	(0.9, 3.6)	0.108			
Renal disease	3.0	(1.7, 5.3)	<0.001			
Liver disease	0.9	(0.4, 2.0)	0.830	2.7	(1.1, 6.4)	0.024
Diabetes	1.0	(0.4, 2.8)	0.980			
Peripheral vascular disease	0.4	(0.1, 1.5)	0.151			
Heart rate, (beats/min)	1.0	(1.0, 1.0)	0.699			
SBP, mmHg	1.0	(1.0, 1.0)	0.071	1.0	(1.0, 1.1)	0.443
DBP, mmHg	1.0	(0.9, 1.0)	0.064	1.0	(0.9, 1.1)	0.888
MBP, mmHg	1.0	(0.9, 1.0)	0.015	1.0	(0.8, 1.1)	0.522
RR, (breaths/min)	0.9	(0.8, 1.0)	0.006	0.9	(0.8, 1.0)	0.075
Temperature, °C	0.6	(0.4, 0.9)	0.005	0.5	(0.3, 1.0)	0.038
SpO_2, %	0.9	(0.8, 1.0)	0.118			
SOFA	1.0	(1.0, 1.1)	0.257			
LODS	1.0	(0.9, 1.1)	0.752			
SAPS II	1.0	(1.0, 1.0)	0.046	1.0	(1.0, 1.1)	0.462
Vasoactive agent	1.5	(0.8, 2.8)	0.219			
Invasive Vent	0.4	(0.2, 0.7)	0.001	0.5	(0.2, 1.2)	0.125
WBC, 10^9/L	1.0	(0.9, 1.0)	0.278			
Hemoglobin, g/dl	0.9	(0.8, 1.0)	0.138			
MCV, fl	1.0	(1.0, 1.1)	0.091	1.0	(0.9, 1.1)	0.894
PLT, 10^9/L	1.0	(1.0, 1.0)	0.046	1.0	(1.0, 1.0)	0.680
RBC, 10^{12}/L	0.7	(0.5, 1.0)	0.040	1.4	(0.2, 10.1)	0.736
Anion gap, mmol/L	1.1	(1.0, 1.1)	0.025	1.0	(0.8, 1.2)	0.931
Bicarbonate, mmol/L	1.0	(0.9, 1.0)	0.382			
BUN, mg/dl	1.0	(1.0, 1.0)	0.119			
Calcium, mg/dl	1.1	(0.8, 1.4)	0.521			
Chloride, mmol/L	0.9	(0.9, 1.0)	<0.001	0.9	(0.8, 1.1)	0.445
Creatinine, mg/dl	1.1	(0.9, 1.2)	0.265			
Glucose, mg/dl	1.0	(1.0, 1.0)	0.100			
Sodium, mmol/L	0.9	(0.9, 1.0)	<0.001	1.0	(0.8, 1.2)	0.950
Potassium, mmol/L	0.9	(0.6, 1.3)	0.464			
INR	1.7	(1.3, 2.2)	<0.001	NA		
PT, s	1.1	(1.0, 1.1)	<0.001	0.6	(0.4, 1.1)	0.109
APTT, s	1.0	(1.0, 1.0)	0.365			
RDW, %	1.3	(1.2, 1.4)	<0.001	1.2	(1.1, 1.4)	<0.001

Note: SAE, sepsis-associated encephalopathy; ICU, intensive care unit; CI, confidence interval; HR, hazard ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; RR, respiratory rate; SOFA, sequential organ failure assessment; LODS, logistic organ dysfunction system; SAPS II, simplified acute physiology score II; WBC, white blood cells; MCV, mean corpuscular volume; PLT, platelet; RBC, red blood cells; BUN, blood urea nitrogen; INR, international normalized ratio; PT, prothrombin time; APTT, activated partial thromboplastin time; RDW, red blood cell distribution width.

Supplementary Table 2. Univariate and multivariate Cox regression model used to study the association of RDW with 30-day mortality in unmatched young SAE patients in ICU

	Univariate analysis			Multivariate analysis		
	HR	95% CI	P-value	HR	95% CI	P-value
Female	1.0	(0.7, 1.5)	0.808			
Race						
White	Ref			Ref		
Black	1.0	(0.5, 1.8)	0.902	1.1	(0.6, 2.1)	0.797
Others	0.5	(0.3, 1.0)	0.056	0.6	(0.3, 1.3)	0.212
Unknown	1.3	(0.9, 1.9)	0.210	2.0	(1.3, 3.0)	0.002
Myocardial infarct	0.4	(0.2, 1.0)	0.055	0.6	(0.2, 1.4)	0.227
Congestive heart failure	0.6	(0.3, 0.9)	0.027	0.4	(0.2, 0.7)	0.002
Chronic pulmonary disease	1.2	(0.7, 1.9)	0.529	0.9	(0.5, 1.7)	0.806
Renal disease	3.3	(2.4, 4.7)	<0.001			
Liver disease	1.0	(0.7, 1.6)	0.877	0.6	(0.2, 1.4)	0.227
Diabetes	0.7	(0.3, 1.5)	0.339			
Peripheral vascular disease	0.4	(0.2, 1.0)	0.055	0.5	(0.3, 0.9)	0.031
Heart rate, (beats/min)	1.0	(1.0, 1.0)	0.869			
SBP, mmHg	1.0	(1.0, 1.0)	<0.001	1.0	(1.0, 1.0)	0.645
DBP, mmHg	1.0	(1.0, 1.0)	0.018	1.0	(1.0, 1.1)	0.750
MBP, mmHg	1.0	(1.0, 1.0)	<0.001	1.0	(0.9, 1.0)	0.440
RR, (breaths/min)	1.0	(0.9, 1.0)	0.014	1.0	(0.9, 1.0)	0.647
Temperature, °C	0.6	(0.5, 0.8)	<0.001	0.6	(0.4, 0.8)	0.002
SpO_2, %	0.9	(0.9, 1.0)	0.036	1.0	(0.9, 1.0)	0.256
SOFA	1.1	(1.0, 1.1)	<0.001	1.1	(1.0, 1.2)	0.157
LODS	1.0	(1.0, 1.1)	0.072	1.0	(0.9, 1.1)	0.787
SAPS II	1.0	(1.0, 1.0)	<0.001	1.0	(1.0, 1.0)	0.853
Vasoactive agent	0.8	(0.6, 1.1)	0.163			
Invasive Vent	0.3	(0.2, 0.5)	<0.001	0.4	(0.3, 0.6)	<0.001
WBC, 10^9/L	1.0	(1.0, 1.0)	0.499			
Hemoglobin, g/dl	0.8	(0.8, 0.9)	<0.001	1.3	(0.9, 1.8)	0.151
MCV, fl	1.0	(1.0, 1.1)	<0.001	1.0	(1.0, 1.0)	0.522
PLT, 10^9/L	1.0	(1.0, 1.0)	<0.001	1.0	(1.0, 1.0)	0.277
RBC, 10^{12}/L	0.5	(0.4, 0.6)	<0.001	0.4	(0.2, 1.2)	0.099
Anion gap, mmol/L	1.1	(1.0, 1.1)	<0.001	1.0	(0.9, 1.1)	0.587
Bicarbonate, mmol/L	1.0	(1.0, 1.0)	0.544			
BUN, mg/dl	1.0	(1.0, 1.0)	<0.001	1.0	(1.0, 1.0)	0.253
Calcium, mg/dl	1.2	(1.0, 1.3)	0.028	1.1	(0.9, 1.3)	0.291
Chloride, mmol/L	0.9	(0.9, 1.0)	<0.001	1.0	(0.9, 1.1)	0.561
Creatinine, mg/dl	1.1	(1.0, 1.1)	0.140			
Glucose, mg/dl	1.0	(1.0, 1.0)	0.067	1.0	(1.0, 1.0)	0.958
Sodium, mmol/L	0.9	(0.9, 1.0)	<0.001	1.0	(0.9, 1.1)	0.625
Potassium, mmol/L	1.0	(0.8, 1.2)	0.884			
INR	1.3	(1.2, 1.5)	<0.001	11.4	(0.8, 157.2)	0.068
PT, s	1.0	(1.0, 1.0)	<0.001	0.8	(0.6, 1.0)	0.090
APTT, s	1.0	(1.0, 1.0)	0.028	1.0	(1.0, 1.0)	0.319
RDW, %	1.2	(1.1, 1.2)	<0.001	1.1	(1.0, 1.2)	0.012
Age, y	1.0	(1.0, 1.1)	<0.001	1.1	(1.0, 1.1)	<0.001

Note: SAE, sepsis-associated encephalopathy; ICU, intensive care unit; CI, confidence interval; HR, hazard ratio; HR, hazard ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; RR, respiratory rate; SOFA, sequential organ failure assessment; LODS, logistic organ dysfunction system; SAPS II, simplified acute physiology score II; WBC, white blood cells; MCV, mean corpuscular volume; PLT, platelet; RBC, red blood cells; BUN, blood urea nitrogen; INR, international normalized ratio; PT, prothrombin time; APTT, activated partial thromboplastin time; RDW, red blood cell distribution width.

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PERSPECTIVE

The future of minimally invasive surgery: A revolutionary new chapter in medicine

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Received December 10, 2025; Accepted February 28, 2026; Published March 31, 2026

DOI: 10.61189/189238vryaia

Abstract

Minimally invasive surgery (MIS) has been developed based on the concept of minimum trauma and maximal function, epitomizing the advancement in modern surgery. Following the introduction of laparoscopy in the 1980s, MIS has continued to evolve into multiple modalities, including single-incision surgery, natural orifice transluminal endoscopic surgery, and robotic surgery. The advancements in imaging, artificial intelligence (AI) and robotization have come together to enhance not only the precision and safety of operations, but also the visualization and technical feasibility even in very complex cases. Numerous investigations have shown that MIS, compared with open surgery, decreases blood loss, reduces complication rates, shortens hospital day, and improves postoperative quality of life. The use of AI has brought surgery to the new age of machine intelligence and data-based decision making. Nevertheless, ethical and legal barriers, inadequate physician training programs, and uneven distribution of resources are obstacles to its wider implementation. The next wave of MIS, will be characterized by intelligence and personalization, incorporating AI navigation, augmented reality, and a multi-disciplinary approach.

Keywords: Minimally invasive surgery, Robot-assisted surgery, Artificial intelligence, Precision surgery

1 INTRODUCTION

Minimally invasive surgery (MIS) is a diagnostic and therapeutic procedure performed through small incisions or natural orifices, aided by image-guided navigation, endoscopy, robotics, and other assistive technologies, with the core aims of reducing surgical trauma, shortening postoperative recovery, and lowering the risk of complications. Laparoscopic cholecystectomy, a landmark development in the 1980s, ushered in a revolutionary era of modern MIS, with single-port laparoscopic surgery later advancing this progress. Recently, new technologies including natural orifice transluminal endoscopic surgery and robot-assisted surgery (RAS) have been developed, marking the transition from open surgery to accurate, minimally-invasive intervention [1]. At present, MIS has been extensively used in general surgery, urology, gynecology, cardiovascular surgery and orthopedics. In the future, robotics, artificial intelligence (AI), big data and augmented reality (AR) will further promote the intelligent development and personalization of MIS. Key challenges include ethical and regulatory issues, clinician training, and regional resource disparities; the safe, standardized, and sustainable development of MIS hinges on integrating technological innovation with institutional building. This paper is primarily intended to guide clinical surgeons,

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perioperative managers, and hospital decision-makers in the introduction and management of MIS technology, providing a comprehensive roadmap for technology evaluation and implementation, as well as a clinical perspective to facilitate collaboration with engineers.

2 TECHNOLOGICAL INNOVATIONS DRIVING THE EVOLUTION OF MIS

2.1 The application of advanced imaging technologies in MIS

The development of imaging technology has greatly supported the accurate performance of MIS. Preoperative high-resolution computed tomography, magnetic resonance imaging and three-dimensional (3D) reconstruction provide accurate delineation of the lesions and adjacent structures. These data can then be used to facilitate personalized surgical planning. Multimodality imaging tools such as ultrasound, fluorescence imaging and AR navigation allow more precise intraoperative localization and better visualization during surgery. A markerless AR system can visually display the actual distance between instruments and blood vessels in real time, thereby greatly minimizing any risk of vascular injury. Endoscopy combined with navigation technology not only maintains the advantages of MIS, but also decreases the complexity of anatomic localization [2].

2.2 The rise and prospects of RAS

RAS, which depends on 3D vision and extremely dexterous robot arms, is completely overhauling the precision and safety of surgical procedures. Single-port robotic systems [i.e., da Vinci SP] combine the best of minimal invasiveness with safety during procedures such as prostatectomy and pelvic lymph node dissection [3]. Single-port cholecystectomy in children also has advantages of less trauma, less bleeding and faster recovery. In complex urological reconstruction, the combination of robotics, fluorescence technology and ureteroscopy makes it possible to achieve excellent anatomical visualization. RAS is moving toward not only routine procedures but also advanced cases and applications in special populations, reflecting an important trend in MIS development [4].

2.3 The application of AI in MIS

MIS generates continuous digital signals, such as endoscopic video, instrument trajectories, and robotic kinematic data, which AI can convert into MIS-adaptive assistance—yet AI models need real intraoperative validation (e.g., bleeding, smoke, lens contamination, camera angle changes) rather than clean benchmark videos. Three AI application layers for MIS are emerging: First, perception enables real-time recognition of key anatomy and dissection planes to prevent misidentification-induced catastrophic injuries (e.g., semantic segmentation in laparoscopic cholecystectomy); second, workflow intelligence enables phase/step detection to standardize communication, anticipate equipment needs, and send context-aware prompts (proven in laparoscopic inguinal hernia repair); third, integrated autonomy-in-the-loop fuses video analysis with robotic kinematics to support camera control, instrument collision avoidance, and safety "no-go" alerts—functions unattainable with rule-based software alone [5]. With the rapid advancement of medical engineering and intelligent technology, traditional laparoscopic surgery is continuously evolving towards intelligence and remote control (**Figure 1**).

3 DUAL ENHANCEMENT OF PATIENT EXPERIENCE AND SAFETY

3.1 The impact of MIS on postoperative recovery

The advantages of MIS are now widely recognized, including reduced incision size, decreased bleeding, and faster recovery. It has been proven that MIS can effectively relieve pain, reduce the infection rate, decrease hospital stay, and speed up rehabilitation. Intraoperative analgesia using an erector spinae plane catheter has been reported to significantly increase the Quality of Recovery-15 questionnaire score within 48 hours after surgery, highlighting the role of evidence-based pain control in patient satisfaction [6].

3.2 Risk reduction strategies for complications in MIS

Management of risks associated with MIS needs to be considered in the context of a 'triple-decker strategy' across the pre-, intra-, and post-operative phases. Preoperative holistic evaluation of predisposing factors and imaging features aids in identifying high-risk factors. Intraoperative localization technology including imaging navigation and near-infrared fluorescence imaging improves the accuracy of localization and provides the best approach, thereby minimizing bleeding and anastomosis-related complications [7]. By promoting preoperative evaluation, optimizing intraoperative techniques and strengthening postoperative surveillance, clinicians can significantly decrease the incidence of complications in all stages of the surgery, which may contribute to improving perioperative safety and overall treatment outcome.

3.3 Quantitative analysis of patient feedback and satisfaction

Studies have demonstrated that in different types of surgery such as biliary procedures and arthroscopy, the MIS group experiences greater satisfaction (pain control, scar appearance and quality of life) than conventional surgeries [8]. Through the implementation of standardized questionnaires and follow-up mechanisms, the patient care experience has been measured in an objective manner, contributing to workflow-focused optimization as well as quality management. MIS also improves postoperative recovery and patient psychological comfort. Clinical research outcomes are summarized for comparison in such areas as post-operative pain, recovery time, rates of complications and patient satisfaction (Supplementary Table 1).

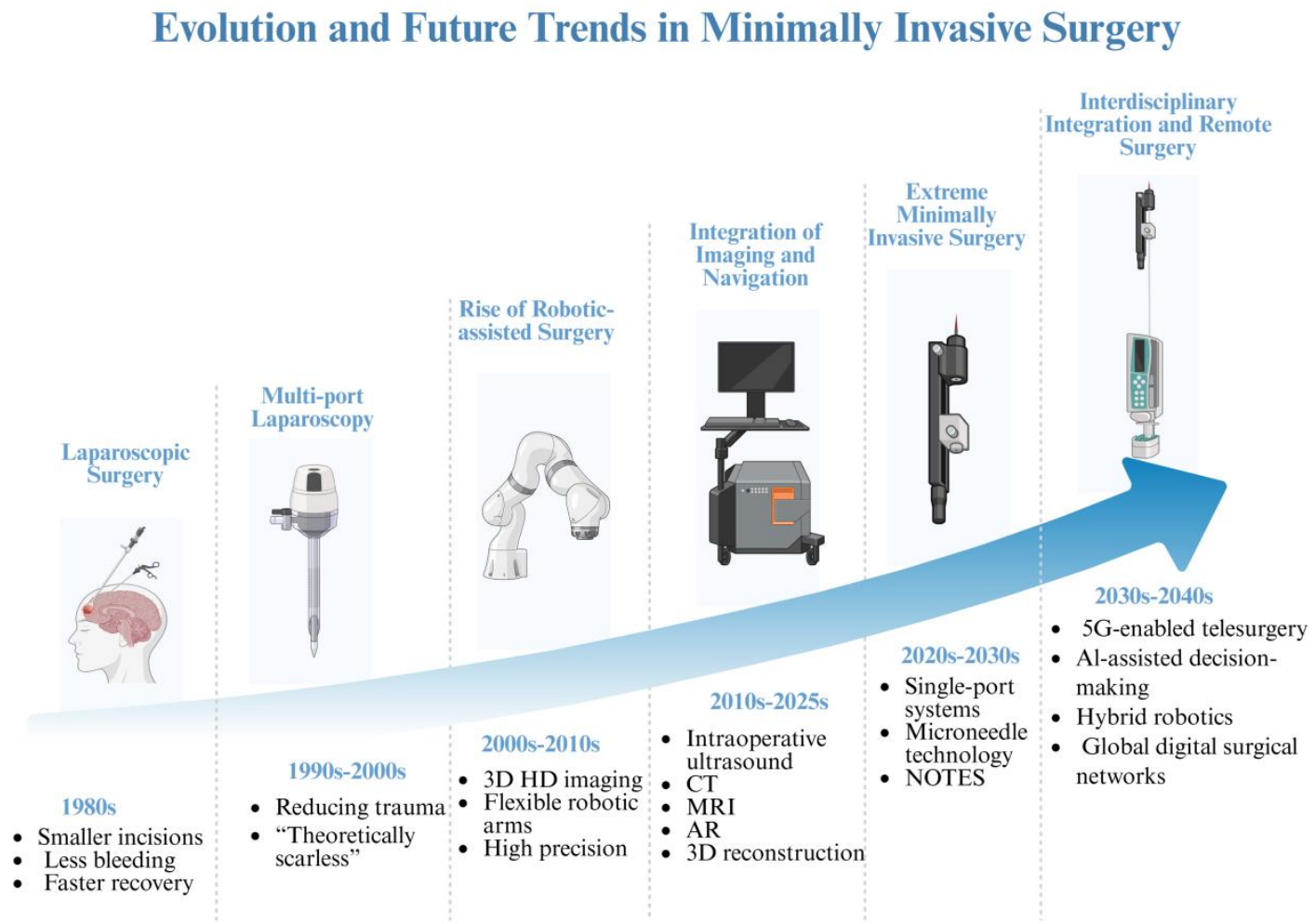

Figure 1. Evolution and future trends of MIS. This figure illustrates the evolution and prospects of MIS technology since the 1980s. Laparoscopic surgery emerged in the 1980s, characterized by small incisions, minimal bleeding, and quick recovery. From the 1990s to the 2000s, multi-port laparoscopic surgery was developed, further reducing trauma. Between 2000s and 2010s, robot-assisted surgery emerged, offering high-definition imaging and high-precision operation. From 2010s to 2025s, the integration of imaging and navigation technologies (such as intraoperative ultrasound, CT, MRI, AR, and 3D reconstruction) has significantly enhanced visualization and accuracy. Looking ahead, from 2020s to 2030s, ultra-MIS is expected to mature, utilizing single-port, microneedle, and NOTES technologies. Finally, from 2030s to 2040s, surgery will enter an interdisciplinary fusion phase, combining 5G remote surgery, AI-assisted decision-making, and a global digital surgical network, thus ushering in a new era of intelligent and remote surgery. 3D, three-dimensional; HD, high-definition; CT, computed tomography; MRI, magnetic resonance imaging; AR, augmented reality; NOTES, natural orifice transluminal endoscopic surgery; 5G, fifth-generation; AI, artificial intelligence.

4 FUTURE CHALLENGES AND STRATEGIES FOR MIS

4.1 Ethical and legal issues in the widespread adoption of technology in MIS

The widespread but heterogeneous adoption of MIS faces several ethical and legal issues, including informed consent, privacy of data, liability in medical malpractice and resource fairness [9]. The risks and long-term durability of new technologies are not always fully understood by patients. If there is a lack of communication, disputes can arise; this underscores the importance of thorough preoperative risk assessment and clear communication. With the growing amount of cross-border imagery and patient data exchange, data protection becomes an important issue that requires comprehensive security and accountability mechanisms to be in place. MIS should be developed in a healthy environment under strong ethical and legal control to guarantee safety and fairness.

4.2 The necessity of physician training and skill enhancement

For MIS, it is extremely necessary for the surgeon to have sufficient experience and systematic training. Systematic, simulation-based, tiered training platforms incorporating animal

experiments and virtual reality can overcome the learning curve, increase surgical accuracy and improve therapeutic outcomes. This emphasizes the role of ongoing education and interdisciplinary cooperation in improving health care quality.

4.3 The impact of healthcare resource allocation and regional disparities

There are economic resource disparities which limit the use of MIS. One of the interesting observations that have emerged from research is that the penetration of MIS is substantially higher in large medical centers than in community hospitals and also varies across patient demographics [10]. For example, Hispanic patients are less likely to accept it [11]. Despite demonstrated more widespread overall utilization of MIS in the surgical staging of endometrial cancer, this practice continues to be applied disproportionately among low-income or vulnerable populations. Notably, shorter hospital stays alone do not equate to full cost-effectiveness, as comprehensive evaluation requires accounting for capital investment, maintenance, consumables, staffing, and long-term clinical outcomes. A practical strategy is to centralize high-cost, advanced MIS platforms in regional hubs and extend their access via referral networks and standardized protocols, thus reducing disparities and ensuring efficient, equitable resource utilization across healthcare settings.

5 DISCUSSION AND OUTLOOK

The next phase of MIS will be defined less by any single device and more by integration: imaging that reduces uncertainty, robotics that extends dexterity, and AI that provides context-aware assistance. 3D reconstruction and markerless AR boost anatomical visualization and intraoperative risk assessment, reducing the impact of varying surgical experience. Single incision platforms and more dexterous robotic arms extend RAS to complex reconstruction and high-risk patients, while AI enables data-driven diagnosis, treatment and postoperative follow-up, shifting from experience-driven practice. In the future, MIS will rely on multidisciplinary cooperation, changing not only surgical philosophy but also the practice model of surgery.

Nevertheless, regional and resource issues remain barriers to its widespread implementation. In developed regions, the utilization rate in central hospitals is significantly higher than that in community hospitals, and there are disparities in patient access owing to inequitable socioeconomic status and insurance coverage. Thus, future development will require not only technology innovation but also policy support, educational training, resource optimization, and improved legal and ethical standards to ensure the safe and equitable application of MIS worldwide. At the same time, specialized training on MIS-related technical skills can be conducted for clinical doctors; a regional referral collaboration model can be established; and remote guidance can be used in appropriate scenarios to comprehensively promote the clinical popularization and application of minimally invasive surgery.

ABBREVIATIONS

AI, artificial intelligence; AR, augmented reality; MIS, minimally invasive surgery.

DECLARATIONS

Author contributions

Yue Shu and Chunyue Jia contributed to the manuscript writing and figure preparation. Shuya Jiang designed the work. Aimin Jiang supervised the work. All authors have read and approved the final manuscript.

Funding

This research received no external funding.

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 with human participants or animals performed by any of the authors.

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 competing interests.

Acknowledgements

Not applicable.

Supplementary Information

The online version (of this article) contains supplementary material available at https://doi.org/10.61189/189238vryaia.

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Supplementary Information

Supplementary Table 1. Comparison of patient experience and safety between MIS and traditional surgery

Dimension	Minimally invasive surgery	Traditional open surgery	Clinical significance	References
Recovery and safety	Video-Assisted Thoracoscopic Surgery (VATS) results in less pain, fewer complications, shorter hospital stays, and better quality of life postoperatively	Thoracotomy is associated with more pain, more complications, longer hospital stays, and poorer quality of life postoperatively	Minimally invasive surgery improves recovery and safety, offering cost-effectiveness	[1]
Recovery and quality of life	Laparoscopic hemihepatectomy offers faster recovery and better quality of life	Open hemihepatectomy leads to slower recovery and lower quality of life	Minimally invasive hemihepatectomy improves recovery and facilitates adjuvant therapy	[2]
Pain and quality of life	Post-VATS pain is lighter, and quality of life is better	Post-thoracotomy pain is more severe, and quality of life is poorer	Minimally invasive surgery reduces pain and improves patient experience	[3]
Recovery and safety	Faster physical recovery and lower complication rates 5 weeks after VATS	Slower recovery and higher rates of severe complications after thoracotomy	Minimally invasive surgery promotes early recovery and safety	[4]
Quality of life and cost	Laparoscopic surgery offers higher Quality-Adjusted Life Years (QALYs) and lower costs	Open surgery results in lower QALYs and higher costs	Minimally invasive surgery improves quality of life and provides significant cost-effectiveness	[5]
Complication occurrence	Laparoscopic surgery has a 30-day complication rate of 27%	Open surgery has a 30-day complication rate of 42%	Minimally invasive surgery offers an acceptable safety advantage	[6]
Quality of life	Laparoscopic surgery results in faster Health-Related Quality of Life (HRQoL) recovery with fewer dimensions	Open surgery results in more significant HRQoL decline and slower recovery	Minimally invasive liver resection improves postoperative quality of life	[7]
Complications and recovery	Thoracoscopic surgery has lower complications, faster recovery, and better quality of life	Open surgery has higher complications, slower recovery, and poorer quality of life	Minimally invasive rib surgery enhances patient experience and safety	[8]
Conversion rate and recovery	Transanal Total Mesorectal Excision (Ta-TME) has a lower conversion to open surgery rate and faster recovery	L-Leucine Antagonist Resistant (L-LAR) has a higher conversion to open surgery rate and slower recovery	Ta-TME improves surgical safety and recovery	[9]
Complications and quality of life	Minimally invasive clipping has better cosmetic outcomes, higher satisfaction, and less temporal atrophy	Wing-point clipping has a 10% facial nerve paralysis rate and poorer quality of life	Minimally invasive clipping improves safety and patient experience	[10]
Immunity and quality of life	VATS has less bleeding, lighter immunosuppression, and better quality of life	Thoracic Outlet Syndrome (TOS) has more bleeding, heavier immunosuppression, and lower quality of life	Minimally invasive lobectomy improves recovery and patient experience	[11]
Pain and recovery	Endovenous Laser Ablation (EVLA)/ Ultrasound-Guided Foam Sclerotherapy (UGFS) results in less pain, faster recovery, and shorter time off work	Open surgery results in more pain, slower recovery, and longer time off work	Minimally invasive methods improve experience and shorten recovery time	[12]
Pain and healing	Sinus tract curettage combined with PRP results in less pain, faster healing, and improved quality of life	Open excision surgery results in more pain, slower healing, and poorer quality of life	Minimally invasive curettage with PRP optimizes recovery and reduces patient burden	[13]
Surgery and recovery time	UGFS+Great Saphenous Vein (GSV) high ligation: 38.3 minutes, recovery in 5.4 days	GSV stripping + multiple point avulsion/TIPP: 81.2 minutes, recovery in 9.6 days	Minimally invasive techniques significantly shorten procedure time and accelerate recovery	[14]
Experience and safety	Laparoscopic surgery results in less pain, higher satisfaction, and fewer side effects	Open surgery results in more pain, lower satisfaction, and more side effects	Minimally invasive techniques optimize experience and improve safety	[15]

Note: Abbreviations: VATS, Video-Assisted Thoracoscopic Surgery; QALYs, Quality-Adjusted Life Years; HRQoL, Health-Related Quality of Life; Ta-TME, Transanal Total Mesorectal Excision; L-LAR, L-Leucine Antagonist Resistant; TOS, Thoracic Outlet Syndrome; EVLA, Endovenous Laser Ablation; UGFS, Ultrasound-Guided Foam Sclerotherapy; GSV, Great Saphenous Vein.

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Expert opinion on perioperative management of bleeding and coagulation in anesthesiology (2026 edition)

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Received September 2, 2025; Accepted September 10, 2025; Published March 31, 2026

DOI: 10.61189/909222ormctb

Abstract

With advancements in surgical techniques and increasing complexity of patients' underlying conditions, the management of perioperative bleeding and coagulation disorders has intensified. Clinical practice faces issues such as non-standardized monitoring, inconsistent management protocols for specific patient populations, and unclear thresholds for blood transfusion and hemostatic agent administration. Based on evidence-based principles and integrating the latest evidence since 2020, the task force developed this consensus, titled "Expert Opinion on Perioperative Management of Bleeding and Coagulation in Anesthesiology (2026 Edition)". Focusing on the entire perioperative process, it covered coagulation assessment and monitoring, indications for blood product transfusion, rational use of hemostatic agents, and individualized strategies for special populations, including patients on antithrombotic therapy and those undergoing cardiac, neurosurgical, orthopedic or obstetric surgery. It emphasized preoperative risk screening, intraoperative dynamic monitoring, and post-operative balance between hemostasis and thrombosis prevention. Clinical decision-making should integrate the patient's baseline condition, surgical type, and real-time monitoring results. This opinion aims to provide standardized, multi-disciplinary guidance, optimize management protocols, reduce transfusion-related risks and thrombotic complications, and ultimately enhance perioperative patient safety and improve outcomes.

Keywords: Perioperative period, Hemorrhage, Blood coagulation, Expert opinion

1 INTRODUCTION

Perioperative hemorrhage and coagulation abnormalities are major challenges in surgical practice. Uncontrolled bleeding can result in significant blood loss, hemodynamic instability, and a cascade of adverse outcomes, including increased morbidity, prolonged hospitalization, and elevated healthcare costs [1]. On the other hand, excessive correction of coagulation deficiencies may lead to thromboembolic events, posing an equally serious threat to patient safety. The perioperative period, therefore, represents a critical window during which balancing hemostasis and thrombosis is essential [2]. Surgical trauma, anesthesia, and the patient's underlying pathophysiological condition often interact in complex ways, frequently causing coagulation disturbances [3]. The management of perioperative bleeding is further complicated by factors such as pre-existing coagulopathies, the use of anticoagulant or antiplatelet medications, and surgical procedures associated with substantial blood loss [4]. Given these challenges, the need for precise, individualized strategies to manage coagulation and hemorrhage has become increasingly urgent.

Recent advances in diagnostic technologies and therapeutic options have revolutionized perioperative coagulation management. Point-of-care diagnostic tools, such as thromboelastography (TEG) and rotational thromboelastometry (ROTEM), enable real-time dynamic assessments of coagulation status [5]. These innovations facilitate point-of-care monitoring and

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goal-directed therapy tailored to each patient's coagulation profile. Furthermore, the development of new pharmacological agents, including antifibrinolytics and specific reversal agents for anticoagulants, has broadened the therapeutic options available to anesthesiologists. "Anesthesiologist's consensus on perioperative hemorrhage and coagulation management" was first published in China in 2020 [6]. The 2026 updated version integrates the latest evidence in perioperative coagulation management since 2020, building upon the previous edition. It addresses issues in clinical practice, such as non-standardized monitoring, inconsistent protocols for special populations, and ambiguous blood transfusion thresholds, offering optimization and supplementation to provide diagnostic and therapeutic guidance for the perioperative medicine field both domestically and internationally. This consensus is published in both Chinese and English to facilitate clinical practice exchanges across regions and further enhance perioperative patient safety and outcomes.

2 METHODS FOR OPINION FORMULATION

2.1 Objectives and scope of the opinion

This opinion aims to establish a standardized, evidence-based framework for the perioperative management of hemorrhage and coagulation disorders. It serves as a comprehensive guide for anesthesiologists and other healthcare professionals involved in perioperative care.

2.2 Target population of the opinion

These recommendations apply to both adult and pediatric surgical patients. Special patient populations include individuals with underlying coagulopathies, those receiving anticoagulant or antiplatelet therapy, obstetric patients, and those undergoing major surgeries, such as cardiothoracic, neurosurgical, and orthopedic procedures.

2.3 Target users of the opinion

The target users are medical personnel involved in the perioperative management of various types of surgeries, including anesthesiologists, surgeons, pharmacists, and nurses.

2.4 Clinical topics

The clinical topics encompass comprehensive strategies for coagulation assessment and monitoring using both traditional laboratory tests and advanced point-of-care diagnostics, the diagnosis and management of coagulation disorders and perioperative bleeding complications, evidence-based protocols for the administration of blood products (including red blood cells [RBCs], platelets, fresh frozen plasma [FFP], and cryoprecipitate), the therapeutic use of hemostatic agents such as antifibrinolytics, procoagulants, and anticoagulant reversal agents, as well as management strategies for mitigating the risk of thrombosis and thromboembolism throughout the perioperative period.

2.5 Opinion development team and process

The consensus panel was carefully selected, comprising 16 experts. A structured three-step Delphi methodology was employed to develop the consensus, conducted between June 2024 and July 2025, with the goal of formulating thorough and practical clinical recommendations. Each panel member independently evaluated the proposed recommendations using a 4-point Likert scale, allowing for a quantitative assessment of the importance of each recommendation. Panel members were also encouraged to propose revisions to enhance clarity and content, ensuring that the recommendations were both practical and accessible.

2.6 Literature search

A comprehensive literature search was conducted to ensure that the recommendations were based on robust evidence. Premier databases such as MEDLINE, Embase, CINAHL, and CENTRAL were searched using key terms, including "perioperative bleeding", "perioperative coagulopathy", "hemorrhage management", "coagulation disorder", "hemostasis", "anticoagulant reversal", "transfusion therapy", "thromboelastography", "massive transfusion protocol (MTP)", and "coagulopathy diagnosis". In addition to database searches, references familiar to the working group and guidelines from national and international bodies were thoroughly reviewed. The working group predominantly relied on systematic reviews, meta-analyses, and randomized controlled trials to generate evidence-based recommendations. However, it was acknowledged that recommendations concerning laboratory and organizational aspects would largely be based on observational studies. In areas with limited evidence, the working group sought to provide practical, actionable advice by leveraging their combined clinical experience to address knowledge gaps.

3 PERIOPERATIVE HEMORRHAGE AND COAGULATION MONITORING

Effective perioperative hemorrhage and coagulation management begins with a comprehensive medical history and thorough physical examination. Key aspects of the medical history should include personal and family history of bleeding disorders to identify potential hereditary predispositions, an assessment of chronic liver or kidney dysfunction given their significant impact on hemorrhage and coagulation dynamics, and a thorough review of current medications, as many pharmaceuticals can interfere with the coagulation cascade.

Physical examination should focus on identifying signs of bleeding disorders, including purpura, ecchymosis, and subcu-

taneous hematomas. Combined insights from medical history and physical examination often offer more value than conventional laboratory tests, such as activated partial thromboplastin time (APTT), international normalized ratio (INR), and platelet count in the preoperative setting. While biochemical immunoassays remain the primary screening tool for preoperative coagulation assessment, genetic testing for hereditary coagulation disorders offers additional safety measures across all perioperative phases. Regarding the vitamin K-dependent coagulation factors (II, VII, IX, and X), which play critical roles in maintaining coagulation homeostasis, clinical attention should be directed toward the supply efficiency of their precursors, especially given the rarity of inactivating mutations [7, 8]. This underscores the importance of a holistic patient assessment as a cornerstone for effective perioperative hemorrhage and coagulation management [9].

Hemorrhage and coagulation monitoring within the perioperative context can be categorized as follows.

3.1 Estimation of blood loss

Quantitative blood loss assessment should employ multiple methods, including examining suction canister contents, evaluating hemostatic gauze saturation, and monitoring surgical drainage tube volumes. These comprehensive evaluations provide crucial data to understand the extent of blood loss and guide subsequent management strategies.

3.2 Monitoring of perfusion and oxygen supply to vital organs

A multimodal monitoring approach is essential, going beyond the simple observation of clinical symptoms and signs. Routine monitoring parameters include blood pressure, heart rate, pulse oximetry, and electrocardiogram recordings. In complex or high-risk cases, additional advanced monitoring techniques may be warranted. These can include: echocardiography to assess cardiac function and perfusion, renal function monitoring via urine output to gauge renal perfusion adequacy, cerebral oxygen saturation monitoring to safeguard cerebral perfusion, arterial blood gas analysis to evaluate gas exchange and acid-base balance, and mixed venous oxygen saturation monitoring to assess tissue oxygen extraction and utilization [10-12]. This comprehensive monitoring enables early detection of perfusion deficits or oxygenation impairments, facilitating prompt interventions to optimize patient outcomes.

3.3 Hemorrhage and coagulation function tests

Hemorrhage and coagulation function testing can be divided into two categories: coagulation cascade evaluation and platelet function analysis. For patients with a documented bleeding history or underlying coagulopathies, preoperative standard laboratory test should include prothrombin time (PT), APTT, INR, fibrinogen (FIB), and D-dimer. These tests help stratify the risk of surgical bleeding and guide preoperative medication optimization. When available, viscoelastic testing should complement conventional assays.

Viscoelastic hemostatic assays (VHA), such as TEG and ROTEM, have become indispensable in modern perioperative care [13]. Integrating VHA into surgical protocols enhances blood product stewardship by establishing evidence-based transfusion thresholds for RBCs, platelets, and plasma during surgery, reducing the overuse of these resources. In settings without VHA access, strict adherence to standard laboratory test with predefined transfusion criteria is essential. During surgery, surgeons must perform real-time visual assessments of the operative field to evaluate coagulation status and identify ongoing surgical bleeding. Notably, VHAs provide superior diagnostic utility in identifying postoperative bleeding causes and guiding anticoagulant therapy.

For patients with suspected platelet dysfunction (e.g., preoperative bleeding history or antiplatelet medication), preoperative platelet count and functional testing are recommended for bleeding risk stratification and medication management. Perioperative platelet monitoring has proven effective in reducing bleeding complications and transfusion requirements in trauma and cardiac surgery patients [2, 14]. While modern techniques such as VHA offer rapid and user-friendly testing, bleeding time measurement is no longer recommended due to its poor reliability from multiple confounding factors.

3.4 Point-of-care testing (POCT)

POCT has become integral to perioperative coagulation management, with several clinically valuable modalities available.

(1) Activated clotting time: Activated clotting time provides rapid bedside coagulation assessment, which is critical during cardiopulmonary bypass (CPB) procedures. Real-time anticoagulation monitoring with activated clotting time helps prevent thrombotic or hemorrhagic events.

(2) TEG and ROTEM: As mentioned earlier, TEG and ROTEM have revolutionized coagulation monitoring by offering dynamic visualization of the entire clotting process, from fibrin formation to clot dissolution. These technologies enable data-driven transfusion decisions and targeted hemostatic interventions during surgeries.

4 INDICATIONS FOR BLOOD TRANSFUSION

4.1 RBCs

A restrictive strategy for RBC transfusion is recommended in perioperative management. For most patients, maintaining a hemoglobin (Hb) concentration greater than 7 g/dL throughout

Table 1. Huaxi perioperative blood transfusion indication score

Points added	Minimum FiO_2 to maintain SpO_2≥95% (%)	Adrenaline infusion rate to maintain essentially normal cardiac output (μg/kg/min)	Core body temperature* (℃)	Angina
0	≤35	Not required	<38	None
+1	36-50	≤0.05	38-40	Occurs with exercise, physical labor, or excitement
+2	≥51	>0.05	>40	Occurs during daily activities or at rest

Note: *Core body temperature is measured at the nasopharynx, oropharynx, tympanic membrane, rectum, or esophagus. Axillary temperature with an additional 0.5 °C is considered core temperature.

the perioperative period is generally appropriate. Specifically, for hemodynamically stable adult patients, an Hb threshold of 7 g/dL is suggested as the reference value for considering transfusion. However, if Hb levels drop to 10 g/dL, a comprehensive evaluation of the overall clinical context is necessary. This evaluation should consider factors such as cardiopulmonary compensatory capacity, the patient's metabolic status, and the presence of active bleeding before deciding on RBC transfusion. For patients undergoing cardiac surgery, a threshold of 7.5 g/dL is recommended, while for those undergoing orthopedic surgery, a target of 8 g/dL is more appropriate [15].

In elderly patients and those with impaired cardiopulmonary function, clinical evaluation should focus on the balance between oxygen supply and demand. The decision to administer RBC transfusions to enhance oxygen-carrying capacity should not be based solely on Hb levels. Instead, it should involve a comprehensive assessment that integrates the patient's overall physiological condition, comorbidities, and the specific demands of the surgical procedure. This holistic approach ensures judicious use of RBC transfusions, optimizing patient outcomes while minimizing the risks associated with excessive transfusion [16].

The West-China-Liu's Score provides a framework for determining both the Hb concentration, at which transfusion should be initiated, and the target Hb level after transfusion [17]. The specific methodologies for applying the West-China-Liu's Score are outlined in **Table 1**. The West-China-Liu's Score comprises four components.

(1) Minimum FiO_2 to maintain SpO_2≥95%: The minimum FiO_2 required to maintain SpO_2≥95% serves as a key indicator of pulmonary functional reserve, as attempting to measure the lowest room-air SpO_2 in patients with poor lung function is clinically unsafe.

(2) Adrenaline infusion rate: The adrenaline infusion rate is defined as the rate of adrenaline (or an equivalent vasoactive agent) required to maintain adequate cardiac output. It is important to note that the specific agent used may vary according to different institutional protocols.

(3) Core body temperature: Core body temperature reflects the level of total body oxygen consumption, providing insight into the patient's systemic metabolic demands.

(4) History of angina: A history of angina is a critical factor to consider, as it reflects the heart's high oxygen demand and its particular sensitivity to imbalances between oxygen supply and consumption. In such patients, the severity of angina is a key clinical consideration.

The West-China-Liu's Score consists of 6 baseline points, with up to 2 additional points from each of four dimensions, yielding a total score ranging from 6 to 10 (scores are capped at 10). The score correlates with Hb concentration levels, ranging from 6 g/dL to 10 g/dL, to determine the RBC transfusion trigger and target. If the final score is equal to or lower than the current Hb concentration, RBC transfusion is not required. If the final score exceeds the Hb concentration, transfusion is indicated, with the required units of RBCs being twice the difference between the score and the Hb concentration. This calculation assumes that one unit of RBCs is derived from 200 mL of whole blood in China, and transfusion typically raises the Hb level by approximately 0.5 g/dL in most adults.

4.2 Platelets

Platelet transfusion plays a crucial role in perioperative management, particularly for patients with thrombocytopenia or platelet dysfunction accompanied by abnormal bleeding [16]. The decision to transfuse platelets is guided by both numerical thresholds and clinical judgment.

(1) Platelet count ≥100×10⁹/L: Platelet transfusion is generally not indicated when the count exceeds this threshold.

(2) Platelet count <50×10⁹/L: Platelet transfusion is strongly recommended. A count below 50×10⁹/L significantly increases the risk of perioperative bleeding, and prophylactic transfusion can effectively reduce this risk.

(3) Platelet count between 50×10⁹/L and 100×10⁹/L: In this intermediate range, the decision to transfuse requires careful consideration of clinical factors. The presence of spontaneous

bleeding or excessive bleeding at the surgical site should be evaluated. If such symptoms are present, platelet transfusion may be necessary to improve hemostatic function.

(4) Intraoperative uncontrolled bleeding: If uncontrollable bleeding occurs during surgery and platelet dysfunction is confirmed, immediate platelet transfusion is essential, regardless of the platelet count. In such critical situations, the priority is to restore effective hemostasis and prevent life-threatening hemorrhage.

4.3 Plasma

FFP is the most commonly used plasma product in the perioperative setting and is essential for correcting coagulation factor deficiencies [16]. The following scenarios outline the primary indications for FFP use.

(1) Abnormal coagulation parameters: FFP is indicated when the PT or APTT exceeds 1.5 times the normal range, or when the INR is greater than 2.0, particularly in the presence of diffuse bleeding at the surgical site. Such abnormalities suggest coagulation factor deficiencies, and active bleeding warrants intervention.

(2) Acute massive bleeding: In cases of significant blood loss, or when large volumes of stored blood or RBCs are transfused (approaching the patient's total blood volume), FFP supplementation is crucial to prevent or correct dilutional coagulopathy.

(3) Coagulation disorders: FFP is essential for patients with congenital or acquired coagulation dysfunction, whether due to genetic predisposition or underlying medical conditions. Addressing coagulation factor deficits is critical to avoid excessive bleeding.

(4) Reversal of warfarin: In urgent situations requiring reversal of warfarin's anticoagulant effects, FFP can be administered at a dose of 5-8 mL/kg.

(5) Prophylactic use in high-risk procedures: For patients with abnormal coagulation undergoing invasive procedures or high-risk surgeries, prophylactic FFP administration should be considered to reduce hemorrhage risk.

After FFP infusion, comprehensive clinical evaluation and repeat coagulation tests are necessary. Additional doses may be required based on the results to ensure optimal coagulation and patient safety.

4.4 Cryoprecipitate

Cryoprecipitate is a valuable hematological product rich in essential components, such as factor VIII, FIB, von Willebrand factor (vWF), fibronectin, and factor XIII. In cases of severe hemorrhage with FIB levels below 1.5 g/L, cryoprecipitate administration is strongly recommended [16]. This underscores the critical role of FIB in the coagulation cascade, as its deficiency can significantly impair hemostasis.

5 HEMOSTATIC AGENTS DURING THE PERIOPERATIVE PERIOD

5.1 FIB

In cases of significant hemorrhage accompanied by a decline in FIB levels or impaired FIB function, the administration of a FIB concentrate is recommended [18]. FIB therapy should be initiated when the plasma FIB concentration is between 1.5 and 2.0 g/L, and TEG or ROTEM testing indicates FIB dysfunction. The initial infusion dose of FIB concentrate typically ranges from 25-50 mg/kg.

5.2 Coagulation factor XIII (FXIII)

If progressive or diffuse hemorrhage persists despite adequate administration of FIB concentrate, and the patient remains in a hypocoagulable state, this may indicate a significant reduction in FXIII activity. In such cases, when FXIII activity is markedly diminished (<60%), it is recommended to administer FXIII at a dose of 30 IU/kg [19].

5.3 Four-factor prothrombin complex concentrate (PCC)

For patients experiencing severe perioperative hemorrhage while on oral anticoagulant therapy (involving coagulation factors II, VII, IX, and X), the administration of PCC in conjunction with vitamin K is recommended [1]. Additionally, genetic testing for VKORC1 and CYP2C9*3 polymorphisms is recommended.

For patients not receiving oral anticoagulants but presenting with hemorrhage and prolonged coagulation times, PCC should be administered at a dosage of 20-30 IU/kg. However, it is important to note that a prolonged PT/INR alone should not serve as the sole criterion for PCC administration, especially in critically ill patients.

For patients prescribed novel oral anticoagulants, such as dabigatran, reversal of the anticoagulant effect may be needed during emergency surgical procedures, interventional maneuvers, or life-threatening hemorrhagic complications. In these situations, the specific antidote Praxbind is the preferred therapeutic option. PCC may also be considered when the reversal effect of other agents is suboptimal. PCC is recommended for reversing the anticoagulant action of Factor Xa inhibitors in urgent clinical scenarios.

5.4 Recombinant activated factor VII (rFVIIa)

rFVIIa is not recommended for prophylactic use due to its potential to increase the risk of thrombosis. However, when conventional surgical and interventional radiotherapeutic approaches fail to achieve hemostasis, or when comprehensive treatment strategies are ineffective, rFVIIa should be considered. It can also be used to address coagulation disorders associated with hypothermia or acidosis. The recommended dosage is 90-120 μg/kg, which may be administered repeatedly if necessary [1].

5.5 Lysine analogues

(1) Tranexamic acid (TXA): TXA is recommended for the prevention and/or treatment of hemorrhage associated with major surgical procedures or fibrinolysis. The recommended dosage is 20-25 mg/kg, which may be repeated or administered as a continuous intravenous infusion at 1-2 mg/kg/h. Prolonged use may increase the risk of seizures [20].

(2) ε-aminocaproic acid: ε-aminocaproic acid has been shown to reduce intraoperative blood loss and transfusion requirements for blood products in cardiac, hepatic, and orthopedic surgeries through perioperative infusion [1].

5.6 Desmopressin (DDAVP)

Desmopressin, a synthetic analog of arginine vasopressin, increases the plasma levels of coagulation factor VIII and vWF, simultaneously enhancing platelet adhesion [21]. However, current evidence does not conclusively support its efficacy in reducing perioperative bleeding or allogeneic blood transfusion requirements in patients without congenital bleeding disorders. Therefore, its use should be reserved for specific scenarios, such as acquired von Willebrand syndrome. Repeated dosing may diminish its therapeutic effects.

5.7 Calcium supplements

Maintaining normal calcium levels (≥0.9 mmol/L) is essential for optimal coagulation. Clinicians should routinely monitor and supplement calcium as needed during the perioperative period to maintain the integrity of the coagulation system.

6 GENERAL PRINCIPLES FOR MANAGING PERIOPERATIVE HEMORRHAGE AND COAGULATION

6.1 Preoperative management

6.1.1 Screening for bleedin

The risk of perioperative bleeding can be assessed using seven predictive factors: hematoma formation (>2 cm), bleeding history, menorrhagia, surgical history, bleeding after tooth extraction or delivery, and family history of abnormal coagulation [22].

6.1.2 Treatment of preoperative anemia

Preoperative anemia should be managed with erythropoietin and other agents. This condition, associated with an elevated risk of allogeneic transfusion and postoperative complications, requires comprehensive evaluation and timely intervention.

Iron deficiency anemia, the most common form of preoperative anemia, should be treated with iron supplementation. The choice and route of iron therapy should be based on the severity of anemia, the preoperative preparation timeline, and individual variations in iron absorption and tolerance. Two types of therapeutic iron exist: inorganic iron (e.g., ferrous sulfate) and organic iron (e.g., iron dextran and ferrous fumarate), with inorganic formulations generally causing more significant side effects.

Oral iron should be taken after meals to minimize gastrointestinal side effects, which are typically mild and manageable. Iron absorption is inhibited by the concurrent consumption of cereals, dairy products, or tea, and enhanced by the intake of fish, meat, or vitamin C. Therapeutic efficacy is evidenced by rising reticulocyte counts (peaking 5-10 days post-initiation), Hb elevation (apparent after two weeks), and normalization of Hb levels (achieved within approximately two months). Iron therapy should continue for 4-6 months after Hb normalization and only be discontinued when serum ferritin levels stabilize within the normal range.

For patients with oral iron intolerance or malabsorption, intravenous iron supplementation should be considered. Available intravenous formulations, including low-molecular-weight iron dextran, ferric carboxymaltose, iron sucrose, and iron isomaltoside, demonstrate comparable effectiveness. Typical dosing involves 1,000-1,500 mg, administered via slow intravenous infusion once or twice daily. Most patients show Hb improvement within three days, with substantial increases observed after two weeks. Although intravenous iron may trigger allergic reactions, severe anaphylaxis remains exceedingly rare [23].

In megaloblastic anemia caused by folate or vitamin B12 deficiency, treatment should focus on correcting the underlying cause while supplementing folic acid or vitamin B12.

6.2 Intraoperative management

6.2.1 Prevention of perioperative hypothermia

Vigilant prevention of perioperative hypothermia should be maintained through proactive warming measures, aiming to preserve a body temperature >36 °C whenever possible.

Temperatures <34 °C may impair platelet function and delay thrombin activation [24].

6.2.2 Management of severe acidosis and anemia

Severe acidosis and anemia should be promptly identified and managed. While pH correction does not immediately reverse acidosis-induced coagulopathy, it requires continuous attention during treatment. Coagulation is significantly compromised at pH <7.10, and reduced hematocrit substantially impairs platelet adhesion and aggregation [25].

6.2.3 Autologous transfusion

Autologous transfusion offers distinct advantages over allogeneic transfusion, including prevention of transfusion reactions, blood-borne infections, and immunosuppression. This method remains the only option for patients lacking compatible donor blood. Salvaged autotransfusion uses blood recovery devices to collect, anticoagulate, wash, and filter blood from surgical sites, body cavities, or postoperative drainage before reinfusion [26]. Strict equipment certification and quality standards must be followed during blood recovery processes. Whenever possible, residual CPB circuit blood should be returned to the patient after the procedure. Coagulation parameters should be closely monitored during large-volume autotransfusion [27].

6.2.4 Development of standardized massive transfusion protocols

Clinical teams should be trained to rapidly recognize major bleeding, activate emergency protocols, and initiate timely interventions [28]. Protocol activation requires clear communication among team members, with designated leaders coordinating management. The team leader may assign specific roles for blood bank coordination and resuscitation.

6.3 Postoperative management

Postoperative care focuses on two critical objectives: maintaining normal coagulation to prevent bleeding complications and implementing appropriate anticoagulation to reduce thrombosis risk. This balanced approach optimizes recovery while minimizing morbidity.

6.3.1 Causes of postoperative coagulation dysfunction

(1) Coagulation factor depletion and consumption: Surgical trauma and physiological hemostatic responses may lead to the consumption of coagulation factors, thereby affecting coagulation function.

(2) Hemodilution: Aggressive fluid resuscitation often results in hemodilution, reducing the concentration of coagulation factors in the circulation.

(3) Hypothermia: Inadequate maintenance of body temperature (<34 °C) can inhibit thrombin activity and platelet aggregation.

(4) Acidosis: Acidosis caused by tissue ischemia, shock, or respiratory failure can impair coagulation factor activity and platelet function.

(5) Pharmacological agents: Medications such as aspirin (irreversibly inhibiting platelet function), clopidogrel (interfering with platelet activation), and low molecular weight heparin (LMWH) (for thromboprophylaxis) may alter coagulation status. Attention should be paid to their cumulative effects, and monitoring should be intensified.

6.3.2 Clinical manifestations of postoperative coagulation dysfunction

The primary manifestation of postoperative coagulation dysfunction is postoperative hemorrhage. In the immediate postoperative period, the characteristic sign of fresh blood permeating through the incision dressings may be observed, along with an increased flow rate in the surgical drainage tube. Patients may exhibit physiological responses such as tachycardia, hypotension, or require vasoactive support to maintain hemodynamic stability. Additional signs may include local petechiae, ecchymosis in surrounding tissues, spontaneous epistaxis, gingival hemorrhage, occult gastrointestinal bleeding, or hematuria [29].

Accurately determining the underlying cause of postoperative hemorrhage is essential for prevention. The surgical procedure itself should be thoroughly assessed, and sophisticated diagnostic techniques (e.g., computed tomography) may be required. Immediate surgical intervention, either through direct hemostasis or interventional embolization, should be initiated without delay, even if overt coagulopathy has not been diagnosed. Swift control of bleeding can prevent further clinical deterioration.

Surgically induced postoperative hemorrhage may develop into more severe coagulopathy if not promptly addressed. However, in many cases, bleeding can be controlled by correcting the surgical issue and providing appropriate symptomatic treatment. Postoperative hemorrhage due to an underlying coagulation disorder requires comprehensive therapeutic strategies, as surgical reintervention alone is insufficient.

6.3.3 Prevention of postoperative venous thromboembolic complications

Venous thromboembolic complications, including deep venous thrombosis and pulmonary embolism, are potentially life-threatening. Understanding the associated risk factors is crucial for implementing effective prophylactic strategies [30].

(1) High-risk factors: hip and knee arthroplasty, complex general surgeries, pelvic or femoral fractures, severe multi-system trauma, and spinal cord damage, all of which result in prolonged immobility, compromised endothelial integrity, and hypercoagulable states.

(2) Moderate risk factors: arthroscopic knee procedures, pregnancy and the postpartum phase (characterized by hormonal fluctuations and venous stasis), malignant tumor (malignancy-related hypercoagulability), chemotherapy (which can affect coagulation function), central venous access devices, and a history of venous thromboembolic events.

(3) Low-risk factors: laparoscopic surgery, obesity, age over 50 years, bed rest exceeding 3 days, prolonged sitting, and lower extremity varicose veins.

Drugs involved in prevention and treatment [31-35]:

(1) Heparin: A fast-acting anticoagulant that can be rapidly neutralized by protamine when reversal of the anticoagulant effect is required, but may predispose patients to bleeding tendencies and thrombocytopenia; coagulation function and platelet counts should be monitored during postoperative use.

(2) LMWH: A commonly used postoperative anticoagulant widely employed in orthopedic and vascular surgery, which can be administered as early as 6 hours after surgery to significantly reduce the incidence of postoperative deep vein thrombosis without increasing bleeding risk. It offers more rapid absorption subcutaneously, higher bioavailability, and lower risks of bleeding and thrombocytopenia compared to unfractionated heparin, but lacks a specific reversal agent.

(3) Thrombolytics: Agents such as streptokinase and urokinase primarily used for emergency management of thromboembolic complications, particularly pulmonary embolism, but associated with a high risk of bleeding complications requiring cautious use.

7 PERIOPERATIVE HEMORRHAGE AND COAGULATION MANAGEMENT IN PATIENTS UNDERGOING SPECIAL SURGERY

7.1 Patients on antithrombotic therapy

An increasing number of patients require perioperative antithrombotic therapy for thrombosis prevention. These patients often have concomitant cardiovascular diseases (such as atrial fibrillation, coronary heart disease, or post-valve replacement surgery) or a history of venous thromboembolism (VTE). Although antithrombotic agents effectively reduce thrombotic risk, they disrupt the body's normal hemostatic balance, significantly increasing the risk of perioperative bleeding. This challenge is further compounded by the dual effects of surgical trauma, which induces both coagulation activation and hyperfibrinolysis, making the paradox between bleeding and thrombosis even more complex.

7.1.1 Commonly used antithrombotic agents in clinical practice

7.1.1.1 Antithrombin drugs

(1) Unfractionated heparin: In cases of severe heparin-related bleeding, protamine infusion is required for reversal.

(2) LMWH: Its half-life is 3-4 hours, and protamine can only partially reverse its anticoagulant effect.

(3) Warfarin: As a vitamin K antagonist (VKA) with a half-life of 2-4 days, its anticoagulant effect takes 5-7 days to fully dissipate after discontinuation. INR is the primary indicator for monitoring its anticoagulant effect, and most surgeries require an INR≤1.4.

(4) Direct oral anticoagulants (DOACs): Classified into direct thrombin (factor IIa) inhibitors and direct factor Xa inhibitors based on their mechanism of action. For procedures with low bleeding risk, DOACs should be discontinued 1-2 days preoperatively. However, for moderate-to-high bleeding risk procedures with a creatinine clearance >50 ml/min, rivaroxaban, apixaban, and edoxaban should be discontinued 3 days prior to surgery, with no requirement for bridging anticoagulation [36, 37]. Specific management strategies should reference the 2024 AHA/ACC/ACS/ASNC/HRS/SCA/SCCT/SCMR/SVM Guideline for Perioperative Cardiovascular Management for Noncardiac Surgery [37].

7.1.1.2 Antiplatelet drugs

(1) Aspirin: Cyclooxygenase inhibitor. Continuation of aspirin therapy is generally acceptable during the perioperative period for most surgical procedures. If preoperative discontinuation is necessary, a 5-7 day cessation period is recommended. Platelet transfusion can be considered for managing aspirin-associated bleeding complications.

(2) Clopidogrel: Adenosine diphosphate receptor inhibitor. Discontinuation of clopidogrel 5-7 days before surgery is critical for patients at elevated bleeding risk. In cases of intraoperative or postoperative clopidogrel-related bleeding, platelet transfusion should be considered as a management strategy.

7.1.1.3 Fibrinolytic drugs

Streptokinase: An exogenous activator of the fibrinolytic system with thrombolytic effects. Treatment depends on selective supplementation of platelets and plasma proteins. Most patients

Table 2. The timing of discontinuation and resumption of antithrombotic drugs

Drug	Discontinuation	Resumption (If bleeding risk is low and the patient has resumed oral intake)
Warfarin	Discontinue 5-7 days before surgery. Monitor PT/INR one day before surgery: If INR>1.4, administer oral (or intravenous) vitamin K to reverse its effect. Surgery can proceed safely when INR≤1.4.	Detection of VKORC1 and CYP2C9*3 genes: Resume warfarin 12 to 24 hours after surgery. Restoring the original anticoagulant effect requires 5 to 10 days.
Dabigatran	For normal or slightly impaired renal function: discontinue 2 to 3 days before surgery. For severe renal insufficiency: discontinue 2 to 4 days before surgery. Prolonged discontinuation may be necessary for surgeries with a high bleeding risk.	Resume when bleeding risk is low. Fast-acting; the original anticoagulant effect is restored within 2-3 hours. If necessary, low doses of dabigatran (110 mg, daily) can be given 2-3 days after surgery to replace bridging therapy with low-molecular-weight heparin.
Rivaroxaban/ Apixaban/Edoxaban	Discontinue 2 to 3 days before surgery. Prolonged discontinuation may be necessary for surgeries with a high bleeding risk.	Resume when bleeding risk is low. These drugs are fast-acting, so resumption should be cautious in patients at high bleeding risk.

Note: PT, prothrombin time; INR, international normalized ratio.

require cryoprecipitate transfusion to replenish FIB, along with platelet transfusion to correct platelet dysfunction.

7.1.2 Perioperative management of antithrombotic drugs

The optimal timing for perioperative discontinuation of antithrombotic drugs remains insufficiently supported by large-scale clinical studies. Current practice relies on empirically derived protocols for medication cessation and reintroduction (see **Table 2**) [32-35]. Laboratory monitoring plays a critical role in guiding the precise management of specific agents, enabling clinicians to assess coagulation status and optimize therapeutic decision-making.

7.1.2.1 Bridging therapy

Bridging therapy involves the administration of short-acting anticoagulants during the cessation of long-acting anticoagulants. This approach is typically indicated for patients on antithrombotic medications in specific circumstances, including a history of cerebral embolism or systemic embolic events within the past three months, after mechanical mitral or aortic valve replacement (particularly aortic valve replacement with additional stroke risk factors), in patients with atrial fibrillation and high stroke risk, in those with a history of VTE in the past three months, in cases with previous long-term use of antithrombotic drugs where thromboembolic events occurred during the withdrawal period, and during a specific interval after coronary stent implantation.

For patients undergoing minimally invasive procedures with negligible bleeding risks, such as cataract surgery, minor dental procedures, and superficial skin interventions, bridging therapy is generally unnecessary. In patients with an elevated bleeding risk, the initiation of bridging anticoagulation requires careful evaluation, particularly during the postoperative period [38].

The commonly used drugs in bridging therapy are heparin and LMWH. LMWH is more frequently used due to its ease of administration and reduced need for frequent coagulation monitoring. Bridging therapy can be implemented preoperatively, postoperatively, or during both phases. For patients receiving LMWH bridging prior to elective, high-bleeding-risk surgery, it is recommended to administer half the total daily dose of LMWH the day before surgery, rather than the full dosage.

For patients requiring interruption of DOACs for elective surgery, perioperative heparin bridging should be avoided. Resuming DOACs more than 24 hours after surgery is preferable to resuming within 24 hours [39].

For patients undergoing VKA therapy who need VKA interruption for elective surgery, heparin bridging should be avoided in the following cases: atrial fibrillation, mechanical heart valve, VKA therapy whose sole indication is VTE, and colonoscopy with anticipated polypectomy. For patients undergoing pacemaker or implantable cardioverter-defibrillator implantation while on VKA therapy, continuation of VKA therapy is recommended, avoiding interruption with heparin bridging [40].

Conventional bridging therapy is generally unnecessary for most patients with atrial fibrillation. However, for those at high risk of thromboembolic events (e.g., CHA_2DS_2-VASc score ≥6), bridging therapy should be considered preoperatively and postoperatively. Bridging therapy begins with the discontinuation of the original antithrombotic drug as its effect wanes. It continues until adequate hemostasis is achieved post-surgery, and the original therapy is resumed, restoring its efficacy to pre-discontinuation levels [32].

For patients with prior VTE, if a procedure is required within one month of the acute episode, bridging therapy should be

Table 3. Effect of different antithrombotic drugs on neuraxial anesthesia

Drug	Perioperative management in surgery with neuraxial anesthesia
Aspirin	Monotherapy: Continue therapy
	With other antiplatelet drugs: Discontinue the antiplatelet drugs
	With heparin or low-molecular-weight heparin: Intraspinal anesthesia is not recommended for patients using heparin or low-molecular-weight heparin therapeutically. Discontinue heparin or low-molecular-weight heparin in patients using them prophylactically
Clopidogrel	The incidence of epidural hematoma is uncertain
	Discontinue the drug 5 to 7 days before surgery
Heparin	Subcutaneous heparin: Epidural catheterization can be performed at least 6 hours after a subcutaneous injection of heparin or when APTT returns to normal range after injection
	Intravenous heparin: Should not be administered until at least 60 minutes after catheterization
	The epidural catheter should be removed 4 to 6 hours after heparin is stopped and after APTT or Activated clotting time reaches normal range [44]
	Resume heparin at least 60 minutes after catheter removal
	Avoid concomitant use of other anticoagulants
Low-molecular-weight heparin	Before intraspinal puncture, catheterization, and catheter removal:
	For the prophylactic dose, stop low-molecular-weight heparin 12 hours or more before the procedure
	For the therapeutic dose, stop it 24 hours or more before
	Delay low-molecular-weight heparin administration after surgery if blood staining is found on the epidural puncture needle during the puncture process
	After intraspinal puncture, catheterization, and catheter removal: Do not resume low-molecular-weight heparin until 4 hours later
Warfarin	Discontinue warfarin 5 days before surgery, ensuring INR<1.5
	Timing for removing the epidural catheter: Ensure INR<1.5 before removal
	Oral warfarin should not be resumed until after the epidural catheter is removed post-surgery
DOACs	All product labels include a boxed warning for intraspinal anesthesia
	Discontinue prior to neuraxial block according to the same timeline used for major surgery

Note: APTT, activated partial thromboplastin time; INR, international normalized ratio; DOACs, Direct Oral Anticoagulants.

implemented preoperatively and postoperatively. If the episode occurred more than one month prior, bridging therapy is typically only needed after surgery [41, 42].

7.1.2.2 The bleeding risk in neuraxial anesthesia

Antithrombotic agents have variable effects on bleeding during neuraxial anesthesia. Given the potential severity of bleeding complications within the spinal canal, such as epidural hematoma, the decision to proceed with neuraxial anesthesia must be made with great caution, guided by a thorough patient evaluation. Neuraxial anesthesia should only be performed by experienced anesthesiologists with precision. Postoperative monitoring of peripheral nerve function is crucial, and any associated complications must be promptly identified and addressed (see **Table 3**) [43, 44].

7.2 Patients undergoing cardiovascular surgery

7.2.1 Preoperative preparation

Patients receiving antithrombotic therapy should strictly follow the contents above for comprehensive coagulation status evaluation and risk mitigation. Patients with chronic right heart failure and concomitant hepatic dysfunction (impaired coagulation factor synthesis) require preoperative optimization through multimodal medical management, including hepatic support and nutritional supplementation. For urgent surgeries in antiplatelet/anticoagulant-treated patients, immediate availability of allogeneic platelet reserves is essential to counteract medication-induced hemorrhagic risks.

7.2.2 Drugs to reduce bleeding

7.2.2.1 Prophylactic use of antifibrinolytic agents

Synthetic agents such as TXA, ε-aminocaproic acid, and aminomethylbenzoic acid share similar mechanisms and effectively reduce perioperative blood loss, transfusion requirements, and reoperation rates in cardiac surgery. TXA is particularly potent (approximately 10 times more effective than ε-aminocaproic acid), significantly decreasing RBC transfusion needs and the incidence of re-thoracotomy.

For low-risk bleeding surgeries (e.g., simple valvuloplasty, valve replacement, and coronary artery bypass grafting), a loading dose of 10 mg/kg TXA followed by a maintenance dose of 1-2 mg/kg/h is advised.

For high-risk bleeding surgeries, a loading dose of 30 mg/kg TXA and a maintenance dose of 16 mg/kg/h are recommended. Studies suggest that a total dose exceeding 50 mg/kg may be associated with increased postoperative seizures [45]. Therefore, it is not advisable to exceed a total dose of 50 mg/kg in non-high-risk bleeding patients.

It is crucial to use antifibrinolytic drugs prophylactically in patients undergoing CPB. Regardless of the selected dosage regimen, achieving an effective blood drug concentration prior to CPB initiation is essential. For TXA, a minimum intravenous dose of 10 mg/kg should be administered before CPB to maintain adequate blood levels during bypass, with discontinuation of the infusion after CPB [14, 46]. Research indicates that high-dose TXA infusion reduces the proportion of patients requiring RBC transfusions in cardiac surgeries with CPB. High-dose TXA infusion also leads to less postoperative blood loss in patients with acute type A aortic dissection undergoing surgical repair. Furthermore, TXA is associated with a lower risk of bleeding in patients undergoing coronary artery surgery, irrespective of the use of CPB (on-pump vs. off-pump) or the concomitant performance of valve replacement or other cardiac procedures [46]. However, TXA is associated with an increased risk of postoperative seizures [47].

7.2.2.2 DDAVP

DDAVP is the only pharmaceutical intervention effective in managing hemorrhage caused by abnormal platelet function after CPB-assisted cardiac surgery. Indications include patients undergoing coronary artery bypass grafting who received antiplatelet agents within seven days preoperatively or required CPB durations exceeding 140 minutes. The recommended regimen is 0.3 μg/kg intravenously, capped at 15 μg for patients weighing <100 kg. Pharmacodynamic studies show that therapeutic effects begin within 1 hour of administration and persist for approximately 6 hours. Optimal timing involves administering DDAVP about 1 hour before CPB weaning, typically during the rewarming phase. In non-CPB surgery, DDAVP should be reconstituted in 100 mL of normal saline and administered via slow intravenous infusion over 15-30 minutes. During CPB surgery, medications should be administered via slow intravenous infusion to avoid hypotension. Repeated administration may reduce efficacy, as DDAVP stimulates rapid synthesis of biologically active coagulation factors from vWF and factor VIII precursors in the body. It is ineffective when administered at the commencement or before surgery [48].

7.2.2.3 FIB concentrate

This agent plays a crucial role in replenishing FIB levels, particularly when FIB depletion contributes to abnormal hemorrhage. Timely administration can restore normal hemostasis and mitigate further bleeding complications.

7.2.2.4 PCC

PCC provides a concentrated formulation of prothrombin and other coagulation factors, effectively managing perioperative coagulation factor deficiencies in cardiovascular surgery. This treatment restores the coagulation cascade balance and reduces the risk of hemorrhage.

7.2.2.5 rFVIIa

It is used for salvage treatment of refractory abnormal bleeding after CPB surgery [49]. A low-dose rFVIIa regimen (20-40 μg/kg) should be administered alongside FIB, FFP, and platelet supplementation. This combined approach provides hemostatic effects, achieving hemostasis while reducing the risk of thromboembolic complications.

7.2.3 Other coagulation management measures

7.2.3.1 Heparin anticoagulation and protamine neutralization

During CPB, maintaining adequate heparin anticoagulation prevents excessive clotting. However, protamine exhibits platelet-inhibitory and anticoagulant properties, so its administration should be carefully controlled to avoid overdosage. The initial protamine dose for heparin neutralization should be calculated as 1.0:0.5 of the total heparin amount present in the body (including that used during CPB). For example, if the total heparin dosage is 40,000 U, the initial protamine dose should be 200 mg. After initial neutralization, intermittent protamine supplementation or continuous infusion via a pump is necessary to achieve an approximate 1:1 ratio of total protamine to heparin by the end of the surgical procedure. From the initiation of protamine administration until 6 hours postoperatively, the residual effects of heparin should be regularly assessed, with protamine administered as needed based on the clinical situation [37, 39].

7.2.3.2 POCT

TEG and ROTEM are critical diagnostic tools for patients with abnormal bleeding manifestations or suspected coagulation disorders. These techniques help identify the etiological factors underlying bleeding, guide therapeutic interventions, reduce the volume of allogeneic blood transfusions during the perioperative phase, and improve patient prognosis [50].

7.2.3.3 Autologous plateletpheresis

Preoperative autologous platelet separation technology allows for the collection of autologous platelets and RBCs using a blood separation apparatus. RBCs are re-infused based on the patient's Hb concentration, while platelet-rich plasma is predominantly re-administered to the patient following heparin neutralization [51].

7.3 Patients undergoing neurosurgical procedures

7.3.1 RBC transfusion

Blood conservation strategies in neurosurgery encompass various components, including indications for RBC transfusion, correction of coagulopathies, acute normovolemic hemodilution, autologous blood salvage, use of antifibrinolytic agents, and avoidance of non-steroidal anti-inflammatory drugs.

Traumatic brain injury (TBI), intracranial neoplasms, cerebral hemorrhage, and other conditions can decrease intracranial compliance, disrupting cerebral blood flow regulation. Cerebral blood flow, Hb concentration, blood oxygen content, and blood viscosity are key parameters requiring meticulous monitoring during perioperative management of neurosurgical procedures.

When Hb concentration declines, normal brain tissue compensates by dilating cerebrovascular vessels and increasing cerebral blood flow to maintain stable oxygen supply. However, this compensatory mechanism is limited. When Hb concentration drops to 5-6 g/dL, cerebrovascular dilation reaches its maximum. Further reductions in Hb concentration lead to a decrease in cerebral oxygen supply, resulting in cerebral anoxia and related symptoms [52].

Multiple factors, including TBI, neurosurgical intervention, cerebral hemorrhage, and reduced cardiac output, can impair the brain's compensatory response to declining Hb concentrations. Therefore, it is not feasible to define a universal compensatory capacity for neurosurgical patients. The indications for RBC transfusion should be based on the pathophysiological changes associated with each neurosurgical disorder and the individual patient's clinical condition. Recent transfusion strategies for patients with brain injury have become a focal point in neurocritical care and neurosurgical anesthesia, requiring a balance between cerebral oxygen supply and transfusion-related risks. Three randomized controlled trials published in 2024—TRAIN, HEMOTION, and SAHARA—examined liberal versus restrictive transfusion strategies for various brain injury subtypes, offering guidelines tailored to specific contexts [53-55].

Current evidence supports personalized strategies that consider brain injury phenotypes, pathophysiological phases, and real-time monitoring metrics, such as cerebral oxygen saturation or jugular venous oxygen saturation. These approaches aim to optimize neuroprotection while minimizing complications, such as transfusion-related acute lung injury or immunomodulatory effects that could worsen brain inflammation.

(1) Subarachnoid hemorrhage

Anemia affects 40% to 50% of patients with subarachnoid hemorrhage. Research shows that an Hb concentration below 9 g/dL is an independent risk factor for brain tissue damage in these patients [56]. Maintaining a postoperative Hb level of 11-12 g/dL may reduce the incidence of symptomatic cerebral vasospasm after surgery. Subarachnoid hemorrhage patients often exhibit significantly reduced intracranial compliance, and aggressive RBC transfusion strategies have been linked to adverse outcomes. The transfusion of aged RBCs may also increase the incidence of unfavorable consequences in these patients [57].

The HEMOTION trial, focused on acute ischemic stroke and intracerebral hemorrhage, found no significant difference in 6-month neurological outcomes between liberal (<10 g/dL) and restrictive (<7 g/dL) transfusion thresholds [53]. Although no overall benefit was observed, survivors in the liberal group showed modest improvements in functional independence, highlighting potential subgroup effects that warrant further exploration.

(2) TBI

Due to the heterogeneous nature of injury mechanisms, components, and severities, TBI patients exhibit varying degrees of attenuation in the brain's ability to compensate for anemia. Research indicates that TBI patients with anemia experience poorer recovery of neurological function at 3 and 6 months post-injury [52]. Moreover, the mortality rate is significantly higher in patients with Hb concentrations <9 g/dL. TBI patients who receive allogeneic RBC transfusions are also predisposed to a higher incidence of infection, mortality, and postoperative neurological dysfunction.

For TBI patients with Hb concentrations ≥10 g/dL, allogeneic RBC transfusion is not recommended. For patients with Hb concentrations <7 g/dL, RBC transfusion should be administered. If the Hb concentration falls between 7 and 10 g/dL, RBC transfusion should be considered based on cardiac and pulmonary reserve capacity. Additionally, transfusion should be considered when the brain tissue partial pressure of oxygen decreases below 20 mmHg (1 mmHg=0.133 kPa) or when cerebral oxygen saturation drops below 60% [58, 59].

The TRAIN trial, focused on TBI, compared a liberal transfusion threshold (Hb <9 g/dL) with a restrictive threshold (<7 g/dL). The liberal strategy resulted in a 10% reduction in the risk of unfavorable neurological outcomes at 6 months (62.6% vs. 72.6%) and a 35% decrease in cerebral ischemic events [55]. These findings suggest that maintaining higher Hb levels may mitigate secondary brain injury by improving cerebral perfusion, particularly in TBI patients with disrupted oxygen metabolism. The SAHARA trial, which examined aneurysmal subarachnoid hemorrhage, did not show superiority of the liberal transfusion strategy (for Hb<10 g/dL) over restrictive strategies (Hb<7 g/dL) regarding 12-month modified Rankin Scale

scores. This emphasizes the need for caution in this group, as hypervolemia may worsen cerebral vasospasm or edema [54].

These findings underscore the complexity of transfusion decision-making in brain injury. The optimal transfusion thresholds depend on injury type, pathological stage, and individualized monitoring. For mixed or moderate-severe TBI, the TRAIN trial supports a 9 g/dL threshold to balance oxygen delivery and reduce ischemic risk. However, for aneurysmal subarachnoid hemorrhage, the neutral results of SAHARA caution against over-transfusion, as the risk of adverse hemodynamic effects may outweigh potential benefits.

7.3.2 Platelet transfusion

It is recommended to maintain a platelet count above 100×10^9/L in patients undergoing neurosurgical procedures. Currently, platelet transfusion guidelines in neurosurgery are primarily based on clinical experience and expert judgment. Furthermore, the significance of platelet function measurements in neurosurgery remains unsubstantiated [60].

7.3.3 Correction of coagulation disorder

Patients with acute craniocerebral trauma frequently develop acute traumatic coagulopathy, defined by INR>1.3, PTT>38 seconds, or platelets $<100\times10^9$/L within 7 days of injury. Acute traumatic coagulopathy occurs in 20.6% of patients and is associated with higher rates of shock, hypothermia/hyperthermia, and coagulation derangement. These patients require more blood products, which correlates with increased in-hospital mortality, longer hospital stays, higher incidences of deep venous thrombosis, seizures, and long-term disability. Limited evidence exists regarding the benefits of FFP and PCC for improving coagulation function and prognosis in these patients [61].

TXA can reduce blood loss and the proportion of patients requiring allogeneic transfusion in neurosurgical procedures; however, it may increase the incidence of perioperative seizures [62-64]. Currently, there is insufficient evidence to determine the optimal dosage of TXA for perioperative management in neurosurgery [65].

The administration of non-steroidal anti-inflammatory drugs during elective craniotomy may increase the risk of postoperative intracranial hemorrhage. Thus, non-steroidal anti-inflammatory drugs should be used cautiously during the perioperative period of brain parenchyma surgery in neurosurgery [66].

7.3.4 Autologous blood transfusion

Autologous blood transfusion includes preoperative autologous blood donation, intraoperative cell salvage, and acute normovolemic hemodilution. The indications and contraindications for autologous blood transfusion in neurosurgery are as follows.

Indications: cerebrovascular surgery with anticipated intraoperative blood loss reaching 20% or more of the patient's blood volume, hemispherectomy, surgery for craniosynostosis, and surgery for closed craniocerebral injury.

Contraindications: surgeries with a contaminated operative field (such as transoral and transnasal approaches, evacuation of intracranial or intraspinal abscesses/other infectious foci, and open craniocerebral trauma surgery); surgeries for malignant tumors. Autotransfusion should be used with caution in patients with meningioma, as there have been multiple case reports of ectopic implantation and metastasis of meningioma cells via blood passage through the pulmonary circulation. For meningiomas located near the cerebral venous sinuses, the risk of tumor metastasis associated with autotransfusion must be weighed against the life-threatening risk of massive hemorrhage. Autotransfusion should only be considered in life-threatening emergencies with massive bleeding, when allogeneic blood is unavailable [67].

7.4 Patients undergoing orthopedic surgery

Blood loss varies considerably across orthopedic procedures. Surgeries involving pelvic fractures, femoral shaft fractures, total knee arthroplasty, and spinal interventions are particularly associated with substantial perioperative hemorrhage. Elective orthopedic surgery patients should follow standard perioperative blood management principles, including correcting preoperative anemia, implementing acute normovolemic hemodilution, utilizing intraoperative autologous blood salvage, maintaining normothermia, applying controlled hypotension, optimizing surgical techniques, and managing postoperative anemia. Restrictive transfusion protocols are recommended to minimize allogeneic blood product exposure.

7.4.1 Trauma orthopedic patients

Traumatic injuries, such as pelvic and femoral shaft fractures, can lead to significant blood loss within a short period, potentially causing shock, acidosis, disruptions to the internal environment, and coagulation dysfunction [18]. Acute coagulation dysfunction induced by trauma should be promptly identified and managed to maintain proper coagulation function. RBC transfusion is indicated for patients with acute massive bleeding, hemodynamic instability, and/or insufficient tissue oxygenation [58, 59]. Early treatment of plasma coagulation factor deficiencies is advisable based on the patient's condition, bleeding type, and specific factor deficiency. Coagulation factor complexes, cryoprecipitates, and plasma can be used. TEG should be employed to identify specific coagulation disorders and guide treatment [58, 59]. Platelet transfusion should be administered to maintain the count above 50×10^9/L, with the

dosage adjusted based on TEG findings [59]. For patients with hemorrhage associated with orthopedic trauma, early administration of antifibrinolytic agents should be considered, with TXA recommended as soon as possible within 3 hours post-injury. Antifibrinolytic treatment should be guided by laboratory test results, and the administration of agents should be discontinued once bleeding is effectively controlled [18, 59]. Early initiation of MTP is recommended for patients with severe trauma and significant bleeding [18]. Physical measures to prevent deep vein thrombosis should be implemented within 24 hours after bleeding control, along with pharmacological thromboprophylaxis [18, 22].

7.4.2 Antifibrinolytic and anticoagulant treatment for orthopedic surgery patients

Orthopedic procedures often involve substantial blood loss, including both visible intraoperative hemorrhage and concealed blood loss due to surgery-induced fibrinolysis, which should not be overlooked [68-70]. Given the high risk of VTE associated with these surgeries, anticoagulation therapy becomes necessary—a process that requires a delicate balance between antifibrinolytic and anticoagulant treatments [13].

TXA should be administered perioperatively to patients undergoing hip and knee replacement surgery, either intravenously or topically. Similarly, intravenous TXA administration is advised perioperatively for patients undergoing spinal surgery [13]. In hip and knee replacement surgeries, anticoagulants should be administered 6-12 hours after bleeding cessation. If significant bleeding persists 12 hours postoperatively, the initiation of anticoagulation may be postponed [61, 66]. Before spinal surgery, thrombosis risk should be assessed using the Caprini score (**Table 4**) [67]. For patients receiving TXA during the perioperative period, the following recommendations regarding anticoagulant administration are proposed: For high-risk patients, anticoagulants should be initiated 12 hours after surgery once bleeding has ceased; For intermediate-risk patients, anticoagulants should be initiated 12 to 24 hours after surgery once bleeding has ceased; For low-risk patients, only basic preventive measures and physical methods are recommended. In high-risk bleeding patients, anticoagulation may be delayed until 24 hours post-surgery or potentially withheld entirely [61, 66]. Generally, anticoagulant medications should be administered for 10-14 days. However, in patients with a high VTE risk post-surgery, the duration may be extended to 15-35 days. When abnormal coagulation or hemorrhagic episodes occur, a comprehensive evaluation of bleeding and thrombosis risks is essential, with medication dosages adjusted or discontinued as necessary.

7.5 Patients undergoing obstetric surgery

7.5.1 Assessment of postpartum hemorrhage (PPH)

PPH is defined as blood loss exceeding 500 mL within 24 hours after vaginal delivery or surpassing 1,000 mL after a cesarean section. However, accurately quantifying blood loss during the perinatal period remains challenging in clinical practice. A comprehensive systematic review suggests that improving the precision of blood loss assessment alone may not be sufficient to prevent or manage PPH effectively [71]. Nevertheless, research has shown that implementing standardized bleeding management protocols and promptly preparing blood products can reduce the need for blood transfusions [72].

7.5.2 Coagulation monitoring

In severe obstetric hemorrhage, PT and APTT often remain normal. Emerging evidence has identified hypofibrinogenemia (<2 g/L) as an early predictor of severe PPH. During active bleeding, POCT should be performed every 45-60 minutes based on the clinical condition of the patient [72].

7.5.3 Component transfusion

The current consensus for RBC transfusion in obstetric patients is to maintain an Hb level above 8 g/dL, with a recommended platelet count of at least 75×10^9/L. Plasma FIB levels should be maintained at relatively high levels, with a threshold exceeding 2 g/L. In the context of MTP, a 1:1:1 ratio of erythrocytes, plasma, and platelets is typically employed. However, for patients with PPH, this ratio may not always be applicable, as there is limited research supporting its efficacy in this population [72].

7.5.4 Intraoperative cell salvage

Recent studies and clinical evidence have demonstrated the safety of intraoperative cell salvage in obstetric patients. Indications include limited blood supply or difficulty in cross-matching; cesarean section with anticipated blood loss exceeding 20% of total blood volume; and patients who decline allogeneic blood transfusions due to religious beliefs or personal preferences. During the procedure, two separate suction devices should be used—one exclusively for amniotic fluid collection. The salvaged blood must be processed through a certified filter before reinfusion. For Rh-negative pregnant women, intraoperative cell salvage is recommended when anti-D immunoglobulin is available [73].

7.5.5 Interventional therapy

Interventional strategies primarily include balloon occlusion and arterial embolization. Preoperative imaging confirms abnormal placentation, allowing for prophylactic vascular occlusion. Before cesarean delivery, an abdominal aortic balloon catheter is inserted via femoral access. Post-fetal extraction, gradual balloon inflation achieves pelvic blood flow occlusion, effectively minimizing surgical field hemorrhage. Incremental inflation helps prevent abrupt hypertension in the

Table 4. Caprini risk assessment model for VTE risk factors

A1 (1 point for each risk factor)	B (2 point for each risk factor)
☐ age 40~59 years	☐ age 60~70 years
☐ planned minor surgery	☐ major surgery (<60 min)
☐ recent major surgery	☐ laparoscopic surgery (>60 min)
☐ obesity (BMI>30 kg/m^2)	☐ previous malignancy
☐ bedridden internal medicine patients	☐ obesity (BMI>40 kg/m^2)
☐ history of inflammatory bowel disease	
☐ edema of lower extremity	
☐ varicosity	
☐ severe lung disease, including pneumonia (within 1 month)	
☐ abnormal lung function (chronic obstructive pulmonary disease)	
☐ acute myocardial infarction (within 1 month)	
☐ congestive heart failure (within 1 month)	
☐ sepsis (within 1 month)	
☐ blood transfusion (within 1 month)	
☐ lower limbs were fixed in plaster or brace	
☐ central venous indwelling catheter	
☐ other risk factors	
C (3 point for each risk factor)	**D (5 point for each risk factor)**
☐ age 75 years or older	☐ stroke (within 1 month)
☐ major surgery lasts 2 to 3 hours	☐ acute spinal cord injury with paralysis (within 1 month)
☐ obesity (BMI>50 kg/m^2)	☐ elective lower joint replacement
☐ history of superficial or deep vein thrombosis or pulmonary embolism	☐ multiple trauma (within 1 month)
☐ family history of thrombosis	☐ hip, pelvic or lower limb fracture
☐ currently diagnosed with malignant tumor or undergoing chemotherapy	☐ major surgery (≥3 h)
☐ thrombocytopenia caused by heparin	
☐ unspecified congenital or acquired thrombophilia	
☐ positive anticardiolipin antibodies	
☐ positive for prothrombin G20210A mutation	
☐ factor V Leiden mutation positive	
☐ lupus anticoagulant positive	
☐ elevated serum homocysteine levels	
A2 for women only (1 point for each risk factor)	
☐ oral contraceptives or hormone replacement therapy	
☐ pregnancy or postpartum (within 1 month)	
☐ history of unexplained stillbirth, recurrent spontaneous abortion (≥3 times), preterm delivery due to preeclampsia or fetal growth restriction	
Total score range: 0 and above	
Risk stratification:	
☐ Low risk: 0 or 1 point	
☐ High risk: 3 or 4 points	
☐ Very high risk: ≥5 points	

Note: VTE, venous thromboembolism; BMI, body mass index.

upper extremities. Post-uterine closure, arterial embolization reduces postoperative bleeding and the need for hysterectomy. Cesarean interventions require vigilant prevention, diagnosis, and management of thromboembolic complications [74].

7.5.6 Amniotic fluid embolism with disseminated intravascular coagulation (DIC)

Classic amniotic fluid embolism manifests as sudden cardiopulmonary collapse, pulmonary hypertension, and coagulation

dysfunction during labor or immediately after delivery. While maintaining respiratory and circulatory function, anesthesiologists should assess coagulation status as early as possible and actively correct coagulation disorders. For patients complicated with DIC, RBCs and coagulation factors should be promptly supplemented according to MTP. In cases of hyperfibrinolysis (particularly hypofibrinogenemia), priority should be given to FIB supplementation, along with early administration of TXA for antifibrinolytic therapy. The hypercoagulable phase of DIC is difficult to identify clinically; therefore, heparin therapy is not routinely recommended unless there is clear evidence of a hypercoagulable state [75].

7.5.7 TXA

Evidence supports the therapeutic equivalence of TXA in PPH to trauma and surgical contexts [76]. It significantly reduces the need for PPH-related surgical interventions (e.g., exploratory laparotomy and hemostatic procedures) and decreases bleeding mortality without increasing thromboembolic risk [77].

8 CONCLUSION AND OUTLOOK

Balanced management of perioperative bleeding and coagulopathy is one of the core pillars for ensuring the safety and improving the prognosis of surgical patients. Its complexity has become increasingly prominent alongside the advancement of surgical techniques, the diversification of patients' underlying comorbidities, and the widespread clinical application of antithrombotic therapy. This expert consensus, based on the latest high-quality evidence-based clinical data accumulated in recent years, establishes a standardized management framework covering the entire perioperative care pathway. With the core logic of "Risk Prediction–Dynamic Monitoring–Precise Intervention–Balanced Protection", this consensus defines the key indicators for preoperative risk screening and correction strategies for anemia, standardizes multimodal protocols for intraoperative blood loss assessment, visceral perfusion monitoring, and coagulation function testing, specifies the transfusion indications and dosages of blood components including RBCs, platelets, plasma, and cryoprecipitate, and clarifies the rational clinical application scenarios of hemostatic agents such as antifibrinolytics, coagulation factor concentrates, and anticoagulant reversal agents. In addition, it provides individualized management strategies tailored to the pathophysiological characteristics of special populations, including patients receiving antithrombotic therapy, and those undergoing cardiovascular surgery, neurosurgery, orthopedic surgery, and obstetric procedures. Perioperative bleeding and coagulation management is rapidly evolving towards precision, individualization, and intelligentization. Going forward, the management system should be continuously optimized on the basis of this current opinion, in parallel with advances in medical technology and the accumulation of clinical research evidence. Furthermore, as perioperative bleeding and coagulation management involves multiple specialties and multiple care links, a routine multidisciplinary team collaboration model should be established in the future, encompassing joint preoperative risk assessment, intraoperative collaborative intervention, and co-management of postoperative complications. Particularly in clinical scenarios including bridging therapy for patients on antithrombotic treatment, initiation and titration of MTPs, and comprehensive prevention and management of thrombotic and hemorrhagic complications, the multidisciplinary team collaboration model should be implemented to achieve seamless integration of the diagnosis and treatment workflow, thereby improving the efficiency and safety of clinical management. Several recommendations in this current opinion are still derived from observational studies. Therefore, more high-quality randomized controlled trials and meta-analyses should be conducted in the future, focusing on key scientific questions including perioperative reversal strategies for DOACs, the optimal dosage and administration timing of antifibrinolytic therapy across different surgical types, and the long-term impact of autologous blood transfusion on special populations (e.g., patients with malignant tumors).

DECLARATIONS

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Funding

This research received no external funding.

Data availability

This expert consensus is not mandatory and shall not serve as a basis for medical accident identification or medical liability determination. It is intended for reference only for relevant healthcare professionals.

Ethics approval and consent to participate

Practice guideline registration: Practice Guideline Registry for Transparency (PREPARE-2025CN1487).

Consent for publication

This article is an authorized English translation of an expert consensus originally published in Chin J Anesthesiol, intended to reach a wider international readership.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

Not applicable.

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Perioperative Precision Medicine

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RESEARCH ARTICLE

Therapeutic potential of Dunhuang Mofeng ointment in rheumatoid arthritis: Integrative network pharmacology and experimental validation

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Received November 11, 2025; Accepted March 16, 2026; Published March 31, 2026

DOI: 10.61189/372252akozze

Abstract

Objective: This study integrated network pharmacology and molecular docking to identify the active constituents, core targets, and potential mechanisms of Dunhuang Mofeng ointment in treating rheumatoid arthritis (RA). The therapeutic efficacy was validated using a collagen-induced arthritis (CIA) rat model. **Methods:** Bioactive compounds and target molecules of Dunhuang Mofeng ointment were identified using R language-based network pharmacological analysis. These were integrated with RA-related differential genes from the GEO database to construct regulatory and protein-protein interaction networks. Gene ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analyses were performed to elucidate intervention mechanisms. Molecular docking simulations validated the conformational stability of core targets. A seven-week CIA rat model was established to assess therapeutic effects, including general health, joint swelling, limb deformities, serum inflammatory markers, and mRNA expression of key targets in joint tissues, thereby validating the network pharmacology predictions. **Results:** The core active ingredients were quercetin, apigenin, luteolin, aloe-emodin, and emodin. Primary therapeutic targets included STAT1, CDK4, CCND1, MCL1, FOS, CDKN1A, and MYC, with anti-inflammatory effects mediated through pathways such as JAK-STAT, PI3K-Akt, and Toll-like receptor pathways. After 7 weeks of treatment, CIA rats showed significant improvements, including reduced joint swelling and deformity, decreased pro-inflammatory cytokines, and increased anti-inflammatory cytokine IL-10. mRNA expression of STAT1, CDK4, CCND1, MCL1, FOS, and MYC in joint tissues was significantly downregulated ($P<0.05$), while CDKN1A expression was upregulated ($P<0.001$). The medium-dose and Western medicine groups exhibited pronounced therapeutic effects. **Conclusion:** Dunhuang Mofeng ointment may exert therapeutic effects in RA by modulating pro-inflammatory cytokines and regulating key genes. These findings support its potential as an effective intervention for RA.

Keywords: Dunhuang Mofeng ointment, Rheumatoid arthritis, Network pharmacology, Molecular docking, Experimental verification

1 INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune disorder marked by chronic synovitis and progressive joint destruction. While primarily characterized by destructive inflammatory synovitis, RA frequently manifests with systemic complications, including skeletal involvement, cardiovascular dysfunction, metabolic disturbances, and cognitive impairments [1, 2].

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Epidemiological data estimate the global annual incidence of RA at approximately 3 cases per 10,000 individuals, with a prevalence approaching 1% [3]. Although the precise etiology of RA remains elusive, existing research highlights the pivotal roles of genetic predisposition and environmental factors in disease onset and progression [4]. Current therapeutic strategies aim to mitigate inflammatory responses, alleviate clinical symptoms, and decelerate disease progression. Disease-modifying anti-rheumatic drugs (DMARDs) represent the cornerstone of RA management, effectively suppressing the inflammatory cascade and preventing further joint structural damage; however, their efficacy and safety profiles warrant further refinement [5].

DMARDs, primarily biologics based on monoclonal antibodies or receptor constructs, target cytokines, cytokine receptors, or cell surface antigens to exert their therapeutic effects. These agents function by inhibiting ligand-receptor interactions, depleting specific cell populations, or disrupting cell-cell communication. However, as protein-based therapeutics requiring intravenous or subcutaneous administration, these DMARDs are inherently limited to extracellular or cell-surface mechanisms, precluding direct modulation of intracellular signaling pathways. This represents a critical constraint in addressing certain intracellular pathological mechanisms [6]. While DMARDs have demonstrated remarkable efficacy in suppressing autoimmune responses and slowing disease progression, their immunosuppressive properties heighten the risk of infections, particularly respiratory and opportunistic infections, especially in immunocompromised patients. Concurrently, the use of oral non-steroidal anti-inflammatory drugs is often restricted by significant dose-dependent adverse effects, including cardiovascular, renal, and hematological toxicities. As a highly heterogeneous condition, RA continues to present significant unmet therapeutic needs, including suboptimal responses to existing treatments, failure to achieve immune homeostasis or complete remission, and the inability to repair damaged tissues. Addressing these challenges requires a more comprehensive understanding of the disease's pathogenesis to overcome its complexity and heterogeneity. Interestingly, treatment preferences among Chinese RA patients reveal a diversified approach. A study involving 18 patients found that 7 utilized DMARDs, 6 transitioned to traditional Chinese medicine (TCM) after discontinuing conventional treatments, and 5 combined DMARDs with TCM therapies [7]. These findings suggest that TCM may offer unique benefits in RA management, not only by alleviating symptoms but also by providing a potential alternative or complementary therapeutic strategy.

The formulation of Dunhuang Mofeng ointment originates from the Dunhuang manuscript *The Second Kind of the Dead Ming's Pulse Classics*. The original recipe, known as the Mofeng Ointment Formula, is preserved in the National Library of France under the catalog number P·3287. The extant fragments of the *Pulse Classics* contain 13 ancient prescriptions, of which 7 are described in detail, with Mofeng Ointment being one of the more thoroughly documented. This historical recipe is not only comprehensive in its composition but also includes meticulous instructions for its preparation and application. The Mofeng Ointment formula consists of seven medicinal herbs, including *Aconitum carmichaelii*, *Salvia miltiorrhiza*, *Zanthoxylum bungeanum*, *Ligusticum chuanxiong*, *Rheum palmatum*, *Croton tiglium*, and *Angelica sinensis*, providing a rich clinical background for its use. As a topical formulation, Mofeng Ointment exerts its therapeutic effects locally by permeating the skin to target the affected area, thereby minimizing systemic absorption and reducing the risk of systemic side effects, particularly those associated with immunosuppression, such as infection. In a similar vein, Voltaren ointment (diclofenac diethylamine cream), also applied topically, is associated with a low incidence of systemic reactions. As a potent inhibitor of prostaglandin synthesis, Voltaren is known for its significant anti-inflammatory and analgesic effects. When applied locally, its active ingredients penetrate the skin to reach the site of inflammation, alleviating both acute and chronic inflammatory responses, particularly those induced by trauma or rheumatism, and providing marked reductions in swelling and pain [8-10]. Given its extensive clinical use, Voltaren ointment was chosen as the treatment control group for the comparative analysis in this study.

In this study, leveraging computer-aided drug design technology and considering the multi-component, multi-target, and multi-pathway characteristics of Dunhuang Mofeng ointment, we systematically explored its potential active ingredients, molecular targets, and associated signaling pathways. The aim was to elucidate the underlying mechanisms through which the ointment intervenes in the pathogenesis of RA. Concurrently, the therapeutic efficacy of Dunhuang Mofeng ointment in RA was validated through animal experiments, with Voltaren ointment serving as the control treatment group for comparative analysis. This study provides a comprehensive investigation of the intervention mechanisms of Dunhuang Mofeng ointment on RA from multiple dimensions, thereby offering a theoretical foundation for its clinical application. Additionally, it presents novel insights and perspectives for advancing the research and development of TCM as an external therapeutic modality. The technical framework of the study is illustrated in **Figure 1**.

2 MATERIALS AND METHODS

2.1 Experimental animal

In this study, 60 specific pathogen-free (SPF)-grade Sprague-Dawley rats (190±10 g, evenly distributed by sex) were procured from SPF (Beijing) Biotechnology Co., Ltd. (License No. SCXK (Beijing) 2019-0010). Ethical approval was granted by Gansu University of Chinese Medicine (SY2023-966). The animals were housed in the SPF-certified animal facility at the Research and Experiment Center of Gansu University of

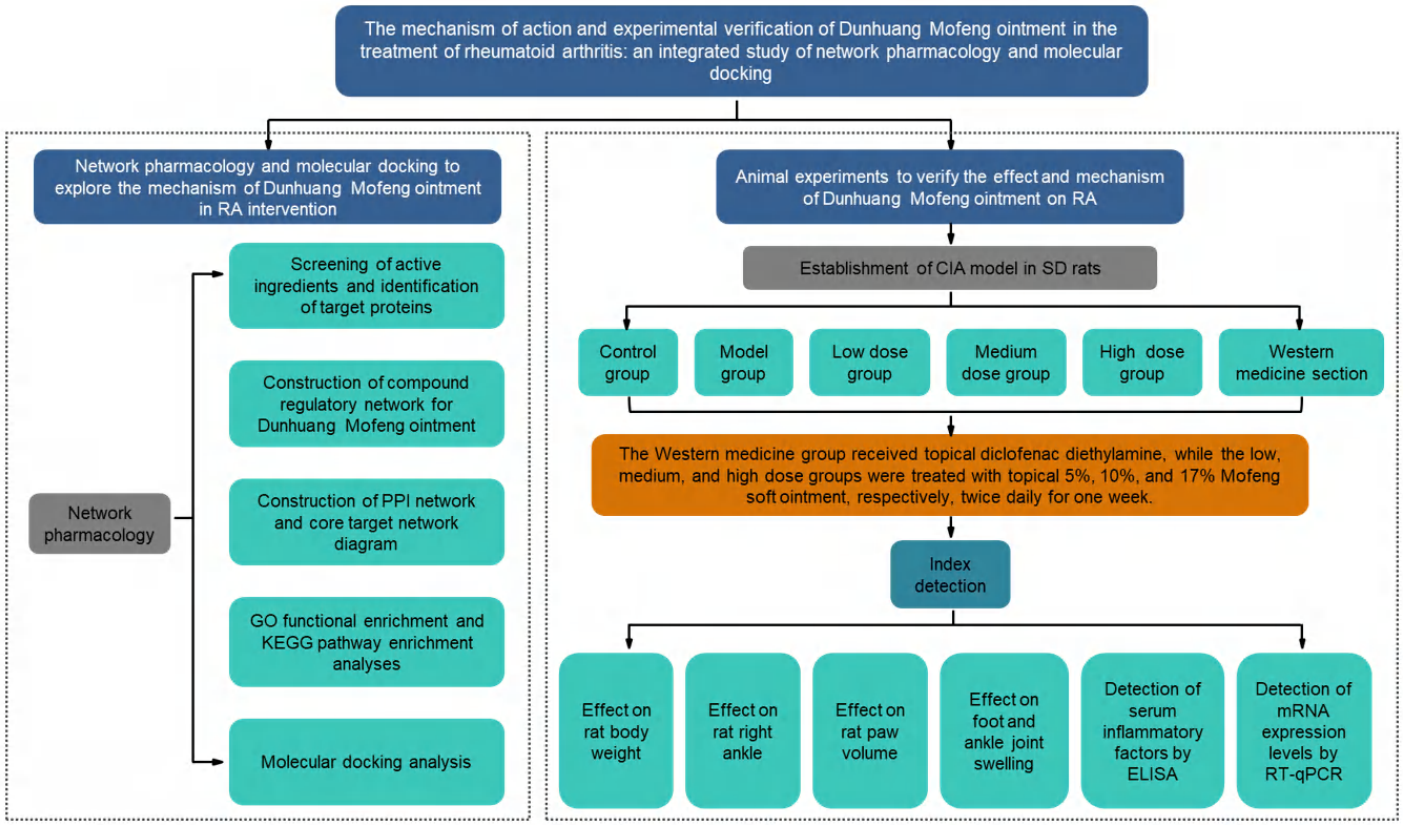

Figure 1. The technical framework of this study. PPI, protein-protein interaction; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; ELISA, Enzyme-Linked Immunosorbent Assay; RT-qPCR, Real-Time Quantitative Polymerase Chain Reaction.

Chinese Medicine under strictly controlled environmental conditions. Temperature was maintained at 23-24 °C, relative humidity at 45-65%, and a 12-hour light/dark cycle was implemented. Throughout the experiment, the rats had ad libitum access to food and water. Following a one-week acclimatization period, the experimental protocol was formally initiated.

2.2 Experimental drugs and reagents

The Dunhuang Mofeng ointment (DHMF2023) used in this study was independently developed by Gansu University of Traditional Chinese Medicine and prepared at concentrations of 5%, 10%, and 17%. These concentrations were selected based on preliminary experimental observations and the commonly used concentration ranges reported in previous studies of topical TCM preparations. Three dose gradients (low, medium, and high) were established to evaluate the potential dose–response relationship of the ointment in the rat model. The control drug, diclofenac diethylamine emulsion (Voltaren), was supplied by GSK Consumer Healthcare SARL (Batch No. EC8P). Key experimental materials included bovine type II collagen (Chondrex, USA; Catalog No. 220241) and complete Freund's adjuvant (CFA) (Sigma, USA; Lot No. SLBR1681V). Enzyme-linked immunosorbent assay (ELISA) kits were provided by Jiangsu Maisha Industrial Co., Ltd., and included the following: Rat TNF-α ELISA Kit (Catalog No. MS-0180R1), Rat IL-10 ELISA Kit (Catalog No. MS-0195R1), Rat IL-1β ELISA Kit (Catalog No. MS-0047R1), and Rat IL-6 ELISA Kit (Catalog No. MS-0190R1). The Bone RNA Extraction Kit was procured from Beijing Biolaibo Technology Co., Ltd. (Catalog No. WM161005). Reverse transcription was performed using the Sunview® III Reverse Transcriptase Kit (with dsDNase, Catalog No. 1334606081), and Quantitative Polymerase Chain Reaction (qPCR) was carried out using the Sunview® SYBR qPCR SuperMix (Universal, Catalog No. 1174624162), both supplied by Shenzhen Shangwei Biotechnology Co., Ltd. Chloral hydrate for experimental use was obtained from Qingdao Yulong Seaweed Co., Ltd. (Batch No. 20120801).

2.3 Establishment of a collagen-induced arthritis (CIA) rat model

A total of 60 Sprague-Dawley rats (30 male and 30 female) were randomly assigned into six groups (n=10 per group, 5 males and 5 females per group) using a random number table method: the control group, model group, low-dose Dunhuang Mofeng ointment group, medium-dose group, high-dose group, and Voltaren group. The CIA model was established in the rats using bovine type II collagen combined with CFA or incom-

plete Freund's adjuvant. For model construction, bovine type II collagen (20 mg) was dissolved in 10 mL of 0.01 mol/L glacial acetic acid, prepared one day prior to the experiment, and stored overnight at 4 °C. On the day of initial immunization, the collagen solution was mixed at a 1:1 ratio with 10 mL of CFA and emulsified thoroughly using an oscillator until a uniform milky state was achieved. For the initial immunization, following intraperitoneal anesthesia with 6% chloral hydrate (6 mL/kg), rats received subcutaneous injections of 0.1 mL of the prepared emulsion at four sites: the dorsal region, 2 cm from the tail base, the lateral side of the right posterior ankle joint, and the right posterior foot. The total injected volume per rat was 0.4 mL. Rats in the control group received equivalent volumes of normal saline administered in the same manner. For the booster immunization, seven days after the initial immunization, a booster emulsion was prepared by mixing bovine type II collagen solution at a 1:1 ratio with incomplete Freund's adjuvant and emulsifying thoroughly. Under anesthesia, rats were injected subcutaneously with 0.1 mL of the emulsion at four sites: the dorsal region, 2 cm from the tail base, the lateral side of the left posterior ankle joint, and the left posterior foot. The total injected volume per rat was 0.4 mL. Model evaluation was performed on the seventh day following the booster immunization. Rats from the blank and model groups were randomly selected for X-ray imaging to assess the success rate of CIA model establishment.

2.4 Administration method and sample collection

Following successful model establishment, drug treatment was initiated, with dosages determined based on the principle of human-to-rat dose conversion as outlined in the Methodology of TCM Pharmacology Research. The experimental groups received the following interventions: the blank and model groups received no treatment; the Voltaren group received external application of Voltaren ointment; the low-dose, medium-dose, and high-dose groups were administered 5%, 10%, and 17% Dunhuang Mofeng ointment, respectively. In accordance with experimental protocols, the corresponding ointment was evenly applied to the ankle joints of the rats in each group. To prevent licking, medical tape was used to secure the ankle joints. The ointment was applied twice daily, between 08:00-09:00 and 20:00-21:00, for a duration of 7 weeks. Following the final dosing, rats were fasted for 24 hours, and samples were collected the subsequent day. Blood was drawn from the abdominal aorta, centrifuged at 3000 rpm for 15 minutes, and the serum was stored at ultra-low temperatures (-80 °C) for subsequent ELISA analysis. After euthanasia by decapitation, the right ankle tissues were rapidly frozen and stored at -80 °C for future qPCR analysis. All experimental measurements, including joint swelling, paw volume, and imaging assessments, were performed by investigators who were blinded to the group allocation.

2.5 Body weight measurement

The rat containment box was placed on an electronic balance and tared to zero. Subsequently, the body weight of each rat in all groups was measured sequentially prior to treatment and on days 7, 14, 21, 28, 35, 42, and 49 following the initiation of drug administration.

2.6 Measurement of right ankle joint diameter

Ankle joint swelling was assessed using a vernier caliper at predefined intervals: prior to treatment and on days 7, 14, 21, 28, 35, 42, and 49 following the initiation of drug administration. Measurements were systematically recorded, with the evaluation points defined as the upper edges of the medial and lateral malleoli.

2.7 Measurement of paw volume

Plantar volume was assessed using a plantar volume measurement instrument at baseline and on days 7, 14, 21, 28, 35, 42, and 49 following drug administration. Methodology: The right ankle joint of each rat was marked with a waterproof marker for consistency. The plantar volume measurement instrument was calibrated prior to use, and the right hind paw of each rat was immersed in a pre-prepared liquid within the measurement container. For each rat, measurements were performed twice, and the average value was recorded for analysis.

2.8 Computed radiography imaging

The right femoral shaft was excised, and computed radiography was performed using a current of 3.2 mA/s, a voltage of 40 kV, and a source-to-detector distance of 90 cm. Radiographic imaging was employed to evaluate callus continuity and fracture healing.

2.9 ELISA detection

Following drug intervention, the serum levels of TNF-α, IL-1β, IL-6, and IL-10 were quantified by ELISA. Serum samples from the treated rats in each group were centrifuged at 3000 rpm for 10 minutes, and the supernatant was carefully collected. Assays were conducted according to the ELISA kit instructions. The optical density at 450 nm was measured, and a standard curve was generated to calculate the concentrations of the relevant cytokines.

2.10 RT-qPCR detection

Total RNA was extracted from rat bone tissue using TRIzol reagent, and samples were stored at -80 °C for subsequent analysis. RT-qPCR was performed using the One Step TB Green PrimeScript RT-PCR Kit to detect the amplified products in

Table 1. Primer sequence information

Gene	GenBank number	Primer sequence	Product/bp
STAT1	NM_007315.4	F: TGTCTGAAGTCCACCCTTCTA R: GTCAAATTCAGAGCCCACTATCT	104
CCND1	NM_053056.3	F: CAACAACTTCCTCTCCTGCTAC R: GAAGGGCTTCAATCTGTTCCT	104
CDK4	NM_000075.4	F: CTTTGGCCTAGCCAGAATCTAC R: ACAGCCAACACTCCACATATC	133
GAPDH	NM_002046.7	F: ACAGCAACAGGGTGGTGGAC R: TTTGAGGGTGCAGCGAACTT	252
MYC	NM_002467.6	F: CTCGGTGCAGCCCTATTTCA R: TAGCGACCGCAACATAGGAC	185
CDKN1A	NM_000389.5	F: CCCGAGAACGGTGGAACTTT R: ACACCAGAGTGCAAGACAGC	231
MCL1	NM_021960.5	F: TTCTCGAGTGATGACCCATGT R: TCCCAGCCTCTTTGTTTCACA	201
FOS	NM_005252.4	F: GAGGGAGCTGACAGATACGC R: TCCAGGGAGGTCACAGACAT	192

real-time. The 20 μL reaction mixture comprised 2 μL of RNA sample, 10 μL of 2× One Step TB Green RT-PCR Buffer, and 0.8 μL of each 10 pmol forward and reverse primers. The reaction conditions included reverse transcription at 42 °C for 5 minutes, followed by denaturation at 95 °C for 10 seconds, and PCR amplification at 95 °C for 5 seconds, 60 °C for 34 seconds, for a total of 40 cycles. PCR products were analyzed using SDS v1.4 software. Gene-specific primers were synthesized by Lanzhou Kebao Co., Ltd. (China). Relative quantification was performed using the 2^−ΔΔCt method with GAPDH as the internal control. Data are presented as mean ± standard deviation (x±s). One-way analysis of variance was conducted using GraphPad Prism 7.0 software. The sequences of the upstream and downstream primers are listed in **Table 1**.

2.11 Extraction of active ingredients from TCM and related targets

In the TCMSP database, the keywords "Rhubarb", "Angelica", "Chuanxiong", "Croton", "Salvia miltiorrhiza", "Aconitum", and "Zanthoxylum" were used for querying. Screening criteria for active ingredient attributes were established based on the transdermal absorption characteristics of topical medications and the five drug classification principles, as follows: molecular weight (MW)≤500, the adipose-water partition coefficient (AlogP) between 1 and 3, and drug-likeness (DL)≥0.18. Active ingredients that met these criteria were selected from the TCMSP database. A file named "ingredients.txt" was created, and "Related Targets" was used to extract the target information for the TCM components. This information was stored in the "targets.txt" file. Using Perl, a script was executed to retrieve the active ingredients of the compound and their corresponding target data, generating a file named "allTargets.txt". The UniProtKB database, with a focus on Reviewed entries, was used to retrieve gene annotation files. The following conditions were applied: Status: Reviewed; Organism: Human. A script was run through Perl to transform the drug target identifiers (IDs) and obtain a file named "allTargets.symbol.txt", thereby constructing the target database for the active ingredients of the drug.

2.12 Identification of overlapping genes between RA-related targets and Dunhuang Mofeng ointment targets

Gene expression data associated with RA were retrieved from the GEO database, including datasets GSE56649, GSE15573, GSE93272, and GSE97779. Following normalization of the genetic data, differential genes were filtered based on |logFC|>1 and an adjusted p-value (adj. P.Val)<0.05. R software was employed to process the differential genes and identify the relevant targets of the active ingredients in Dunhuang Mofeng ointment. The intersection of these genes was then determined, enabling the preliminary identification of potential active ingredients and target proteins for the intervention of RA with Dunhuang Mofeng ointment.

2.13 Construction of the regulatory network diagram for Dunhuang Mofeng ointment

The active ingredients, targets, and RA-related differential genes of Dunhuang Mofeng ointment were systematically screened. Files, including "text file", "net.type.text", "net.geneLists.text", and "net.molLists.text", pertinent to the network regulation of TCM compounds, were generated by executing the Perl script. These files were subsequently imported into Cytoscape 3.8.0 software to construct the regulatory network diagram illustrating the "drug–active ingredient–target" interactions.

2.14 Construction of the protein-protein interaction (PPI) network and core target network diagram

The "disease-drug" common targets were imported into the STRING database, with the following settings: species set to *Homo sapiens* and a medium confidence threshold of 0.400. All other parameters remained at their default values, with free genes excluded. The preliminary PPI network was generated and exported as a TSV file for subsequent analysis in Cytoscape 3.8.0. Network topology analysis and visualization were conducted using the CytoNCA plugin, with the identification of the PPI network core based on metrics such as betweenness centrality, closeness centrality, degree centrality, eigenvector centrality, local average connectivity, and network centrality. The relevant core target proteins were filtered based on scores greater than the median by executing an R script on the resulting data.

2.15 Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis

The R script was executed to convert the symbols of the intersection genes between TCMtargets and RA into gene IDs. The following script parameters were applied: P-value filtering condition: pvalueFilter <0.05; corrected P-value filtering condition: qvalueFilter <0.05. GO functional enrichment and KEGG pathway enrichment analyses were performed. The GO analysis focused on the top 10 biological processes, cellular components, and molecular functions. KEGG pathway enrichment analysis highlighted the top 30 signaling pathways. Visualization was generated using histograms and bubble plots.

2.16 Molecular docking to validate the potential targets and active ingredients of Dunhuang Mofeng ointment in intervening RA

The two-dimensional structures of the small molecule ligands—luteolin, apigenin, quercetin, aloe-emodin, and emodin—representing the top five active ingredients identified in the previous analysis, were downloaded in SDF format from the PubChem database. Using Chem3D software, these structures were optimized and converted into three-dimensional models, which were subsequently saved in MOL2 format. Protein IDs corresponding to the core targets CCND1, MYC, CDKN1A, FOS, MCL1, STAT1, and CDK4 were retrieved from UniProt, and their three-dimensional structures were obtained from the Protein Data Bank (PDB). The receptor proteins were processed to remove water molecules, and small molecule ligands were prepared using PyMOL software. The protein receptor files were saved in PDB format. Hydrogens were added to the protein receptor files using AutoDockTools, and both the protein receptor (PDB format) and the small molecule ligands (MOL2 format) were converted into PDBQT format. To define the active pocket, a Grid Box was generated in AutoDockTools, with the center coordinates of the grid program being adjusted based on the receptor's binding site. Since the exact binding site was unknown, the size of the active pocket was optimized to allow maximum movement of the receptor and ligand during initial docking. The lattice spacing was set to 1 Å, and the dimensions (X, Y, Z) of the grid were determined. The center of the grid was placed at the presumed central region of the binding site, and all relevant parameters were saved in a grid.gpf file. Molecular docking was performed using AutoDock Vina software, with binding energy serving as the key parameter for evaluating ligand-receptor interactions. A binding energy threshold of ≤-5.0 kcal/mol was considered indicative of favorable binding. The docking results were visualized in both 2D and 3D using PyMOL and Discovery Studio software.

3 RESULTS

3.1 Effect of Dunhuang Mofeng ointment on ankle joint swelling in rats from different experimental groups

Compared to the control group, both the model and drug treatment groups exhibited bilateral ankle swelling, reduced mobility, and hair discoloration during both the primary and booster immunizations. On day 7 following the booster immunization, two rats from the control group and two from the model group were randomly selected for X-ray imaging (**Figure 2A-D**). The results revealed pronounced soft tissue edema in the bilateral ankle joints of the model group, with a marked narrowing of the ankle joint space, blurred joint boundaries, and evident bone destruction. Additionally, the appearance of the rats' feet was photographed prior to treatment and at subsequent time points (days 7, 14, 21, 28, 35, 42, and 49 post-treatment) for all experimental groups (**Figure 2E-J**). Compared to the control group, swelling and erythema of the ankle joint extending to the toe joint were observed in the other groups at various time points. In the model group, redness and swelling in the foot and ankle joints progressively worsened from days 7 to 35 post-administration, accompanied by joint deformity, stiffness, and restricted movement. In contrast, the Dunhuang Mofeng ointment (low, medium, and high-dose) groups and the Voltaren group showed a reduction in ankle and foot swelling and a noticeable decrease in erythema after 21 days of treatment. As the treatment period continued, swelling in the ankles and toes of rats in the drug groups gradually diminished. By day 49, joint redness and swelling were nearly completely resolved, with a significant improvement in joint mobility. These findings suggest that both Dunhuang Mofeng ointment and Voltaren have potential efficacy in alleviating symptoms of RA in this model.

3.2 Effect of Dunhuang Mofeng ointment on the body weight of rats in different experimental groups

Observation and analysis of body weight changes in rats across different experimental groups (**Figure 3A**; **Table 2**) revealed a normal growth trajectory in both the model and drug-treated groups prior to administration, with no significant differences compared to the control group. Throughout the entire experimental period (from pre-administration to day 49), the body weight of rats in all groups exhibited a consistent upward trend without any abnormal fluctuations. These findings indicate that the administration of Dunhuang Mofeng ointment did not exert any discernible effects on the body weight of the rats, demonstrating its favorable safety profile.

3.3 Effect of Dunhuang Mofeng ointment on the right ankle joint of rats in different experimental groups

The changes in the diameter of the right ankle joint across the experimental groups are presented in **Figure 3B** and **Table 3**.

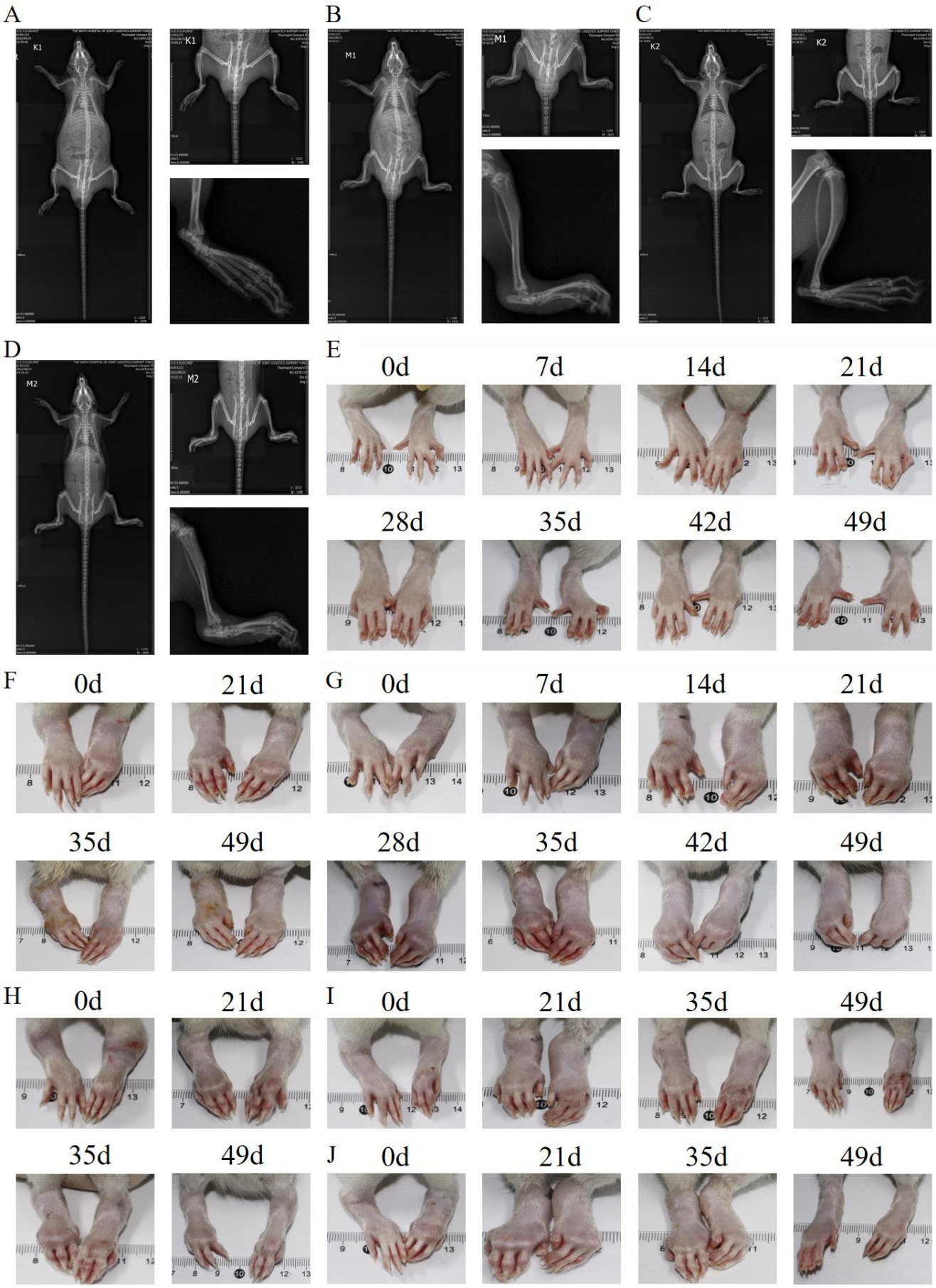
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Figure 2. Radiographic changes and ankle joint swelling in rats across experimental groups. (A-D) Radiographic manifestations of rat ankle joints: A and C: control group, B and D: model group; (E-J) Swelling of the ankle joints in rats at different time points in each group: E: control group, F: high-dose drug intervention group, G: model group, H: Western medicine group (Voltaren), I: low-dose drug intervention group, J: medium-dose drug intervention group.

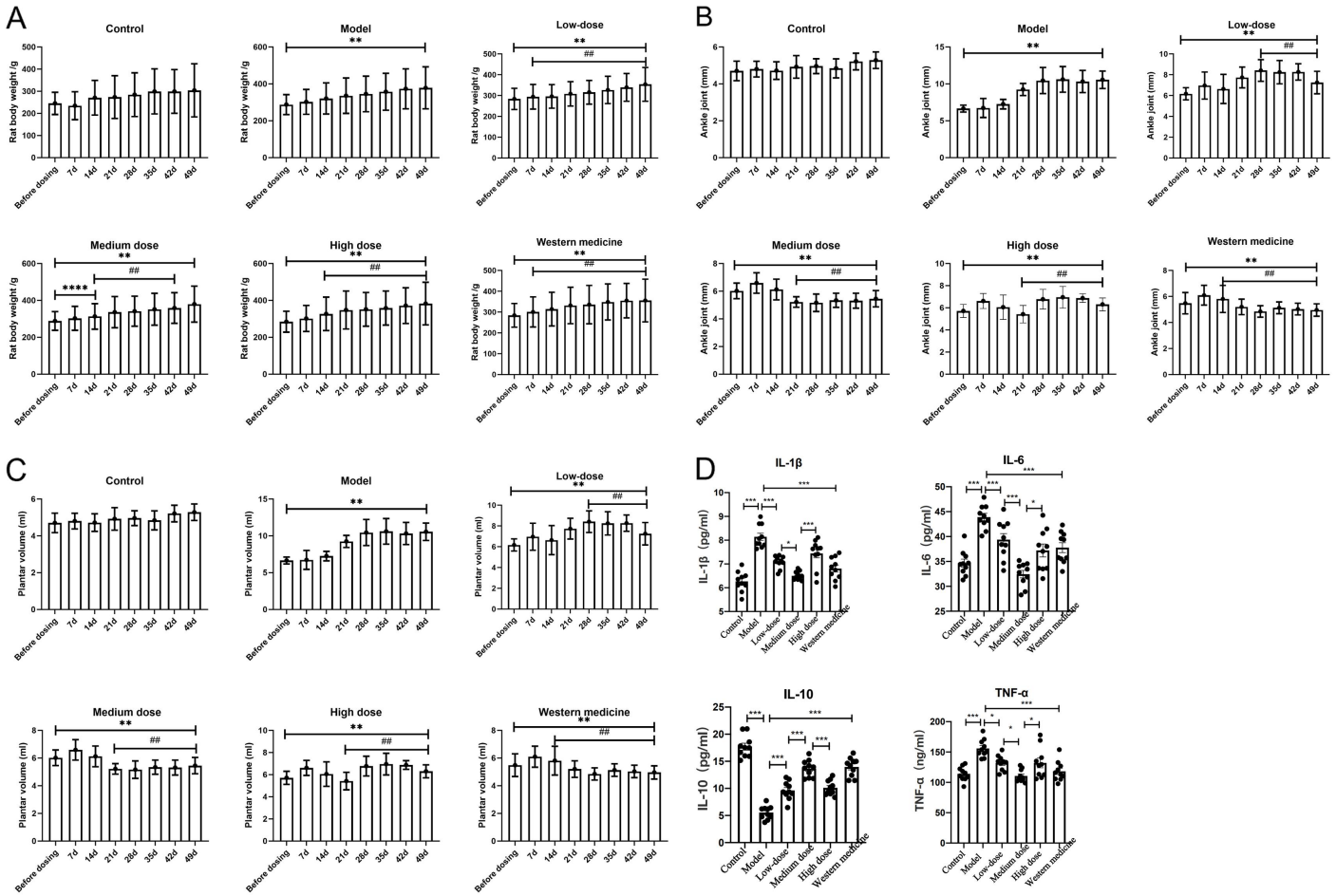

Figure 3. Effects of Dunhuang Mofeng ointment on body weight, ankle joint swelling, plantar volume, and serum inflammatory factors in rats. (A) Influence of Dunhuang Mofeng ointment on body weight of rats in different experimental groups; (B) Changes in the right ankle joint of rats; (C) Changes in plantar volume of rats; (D) Serum inflammatory factor content of rats in each group. Table: *P<0.05, **P<0.01 compared with blank group; $^{\#}$P<0.05, $^{\#\#}$P<0.01 compared with model group. Bar chart: *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

Table 2. Body weight changes in rats across experimental groups (g, mean ± SD, n=10 per group)

Time (days)	Control	Model	Low-dose	Medium-dose	High-dose	Western medicine
Pre	245.10±50.24	287.60±54.25**	283.98±50.69**	288.39±50.69**	284.37±56.80**	284.52±56.76**
7	234.40±63.13	302.31±67.44**	294.27±59.10$^{**,\#\#}$	302.71±64.91**	301.95±70.92**	300.79±72.21$^{**,\#\#}$
14	270.50±78.39	320.80±84.52**	296.01±56.54$^{**,\#\#}$	313.02±69.26$^{****,\#\#}$	326.83±90.72$^{**,\#\#}$	314.42±80.68$^{**,\#\#}$
21	273.50±96.64	335.76±96.08**	308.40±58.42$^{**,\#\#}$	336.26±84.49$^{**,\#\#}$	347.74±102.88$^{**,\#\#}$	331.86±87.58$^{**,\#\#}$
28	284.20±98.77	345.30±96.54**	315.89±56.89$^{**,\#\#}$	341.43±81.27$^{**,\#\#}$	351.54±91.14$^{**,\#\#}$	335.97±92.07$^{**,\#\#}$
35	299.10±101.85	357.36±100.13**	326.93±65.65$^{**,\#\#}$	351.9±86.27$^{**,\#\#}$	358.38±92.34$^{**,\#\#}$	348.73±86.62$^{**,\#\#}$
42	299.00±98.86	373.04±108.13**	339.27±66.62$^{**,\#\#}$	358.69±82.85$^{**,\#\#}$	371.54±96.93$^{**,\#\#}$	355.18±82.69$^{**,\#\#}$
49	304.00±120.11	378.48±113.98**	353.79±81.15$^{**,\#\#}$	379.91±97.45**	382.79±115.43$^{**,\#\#}$	355.59±102.52$^{**,\#\#}$

Note: SD, standard deviation; **P<0.01, ****P<0.0001 compared with control group; $^{\#\#}$P<0.01 compared with model group.

Prior to treatment, the diameter of the right ankle joint in the model and all drug-treated groups was significantly increased compared to the control group (*P<0.01*), indicating the successful establishment of the model. In the model group, foot swell-

Table 3. Changes in right ankle joint diameter in each experimental group (mm, mean ± SD, n=10 per group)

Time (days)	Control	Model	Low-dose	Medium-dose	High-dose	Western medicine
Pre	4.70±0.53	6.65±0.47**	6.17±0.58**	6.02±0.56**	5.71±0.59**	5.48±0.82**
7	4.80±0.43	6.73±1.29**	6.96±1.30**	6.59±0.74**	6.61±0.68**	6.10±0.76**
14	4.72±0.48	7.25±0.64**	6.64±1.40**	6.12±0.75**	6.05±1.10**	5.81±1.04$^{**,\#\#}$
21	4.92±0.61	9.24±0.82**	7.74±1.00**	5.22±0.38$^{**,\#\#}$	5.42±0.79$^{**,\#\#}$	5.20±0.60$^{**,\#\#}$
28	4.96±0.40	10.45±1.77**	8.41±1.04$^{**,\#\#}$	5.16±0.63$^{**,\#\#}$	6.77±0.89$^{**,\#\#}$	4.85±0.44$^{**,\#\#}$
35	4.84±0.52	10.61±1.74**	8.25±1.12$^{**,\#\#}$	5.34±0.51$^{**,\#\#}$	6.95±0.98$^{**,\#\#}$	5.13±0.45$^{**,\#\#}$
42	5.21±0.45	10.32±1.50**	8.27±0.78$^{**,\#\#}$	5.31±0.54$^{**,\#\#}$	6.87±0.39$^{**,\#\#}$	5.03±0.45$^{**,\#\#}$
49	5.28±0.45	10.55±1.18**	7.25±1.08$^{**,\#\#}$	5.45±0.59$^{**,\#\#}$	6.30±0.59$^{**,\#\#}$	4.96±0.48$^{**,\#\#}$

Note: SD, standard deviation; **P<0.01 compared with control group; $^{\#\#}$P<0.01 compared with model group.

Table 4. Changes in right paw volume in each experimental group (mL, mean ± SD, n=10 per group)

Time (days)	Control	Model	Low-dose	Medium-dose	High-dose	Western medicine
Pre	1.76±0.25	2.54±0.41**	2.54±0.34**	2.62±0.44**	2.65±0.30**	2.65±0.26**
7	1.66±0.30	3.37±0.67**	3.37±1.10**	2.98±0.47$^{**,\#\#}$	3.03±0.46$^{**,\#\#}$	3.01±0.35$^{**,\#\#}$
14	1.70±0.41	3.50±0.41**	3.76±1.13$^{**,\#\#}$	3.08±0.58$^{**,\#\#}$	3.03±0.56$^{**,\#\#}$	2.98±0.49$^{**,\#\#}$
21	1.70±0.50	3.16±0.44**	3.35±0.95$^{**,\#\#}$	2.97±0.55$^{**,\#\#}$	3.02±0.60$^{**,\#\#}$	2.89±0.52$^{**,\#\#}$
28	1.59±0.44	2.99±0.29**	3.21±0.76$^{**,\#\#}$	2.86±0.47$^{**,\#\#}$	3.01±0.71**	2.76±0.57$^{**,\#\#}$
35	1.71±0.42	3.05±0.51**	2.78±0.64$^{**,\#\#}$	2.83±0.49$^{**,\#\#}$	2.85±0.65$^{**,\#\#}$	2.66±0.44$^{**,\#\#}$
42	1.79±0.33	3.04±0.54**	3.16±0.67$^{**,\#}$	2.80±0.42$^{**,\#\#}$	2.97±0.72$^{**,\#\#}$	2.50±0.43$^{**,\#\#}$
49	1.77±0.36	2.94±0.52**	3.06±0.73$^{**,\#}$	2.85±0.55$^{**,\#\#}$	2.99±0.65**	2.80±0.55$^{**,\#\#}$

Note: SD, standard deviation; **P<0.01 compared with control group; $^{\#}$P<0.05, $^{\#\#}$P<0.01 compared with model group.

ing progressively worsened, with the right ankle diameter remaining markedly higher than that of the control group at day 35 post-administration (*P<0.01*). Although a gradual reduction in ankle diameter was observed in the model group by day 42, the difference from the control group remained statistically significant (*P<0.01*). In contrast, the drug-treated groups exhibited a significant reduction in ankle joint diameter by day 28 (*P<0.01*), indicating that both Dunhuang Mofeng ointment and Voltaren effectively alleviated ankle joint swelling and mitigated inflammation-related symptoms.

3.4 Effect of Dunhuang Mofeng ointment on paw volume in rats from different experimental groups

The changes in right plantar volume across the experimental groups are illustrated in **Figure 3C** and **Table 4**. Prior to treatment, the right plantar volume in the model group and all drug-treated groups was significantly elevated compared to the control group (*P<0.01*), further supporting the successful establishment of the model. During the treatment period, the right plantar volume in the model group exhibited a slight reduction by day 21, but it remained significantly higher than that of the control group (*P<0.01*). In contrast, all drug-treated groups demonstrated a downward trend in plantar volume by day 14 of administration; however, significant differences from the control group persisted (*P<0.01*). These findings suggest that both Dunhuang Mofeng ointment and Voltaren contributed to the alleviation of foot swelling, although complete resolution to normal levels was not achieved.

3.5 Effect of Dunhuang Mofeng ointment on inflammatory factors in rats from different experimental groups

ELISA analysis of serum biomarkers in rats across the experimental groups (**Figure 3D**) revealed that, compared to the control group, serum levels of TNF-α, IL-1β, and IL-6 were markedly elevated in the model group (*P<0.001*), while IL-10 levels were significantly reduced (P<0.001), confirming the successful induction of a robust inflammatory response. In contrast, treatment with both Dunhuang Mofeng ointment and Voltaren resulted in significant reductions in the serum levels of TNF-α, IL-1β, and IL-6 (*P<0.05*), alongside a notable increase in IL-10 expression (*P<0.001*) when compared to the model group. These results suggest that both treatments effectively modulate immune responses and mitigate inflammation.

3.6 Active components of TCM and their corresponding targets

In the TCMSP database, a total of 76 active ingredients were selected based on the screening criteria of "MW≤500, AlogP: 1-3, DL≥0.18". These included 13, 10, 1, 1, 39, 8, and 4 components derived from *Rheum officinale*, *Angelica sinensis*, *Ligusticum chuanxiong*, *Croton tiglium*, *Salvia miltiorrhiza*, *Zanthoxylum bungeanum*, and *Aconitum carmichaelii*, respec-

Table 5. Active ingredients of Dunhuang Mofeng ointment

Herb	MOL ID	Molecule name	MW	AlogP	DL
Rheum officinale	MOL001729	Crysophanol	254.25	2.76	0.21
	MOL002235	Eupatin	360.34	1.99	0.41
	MOL002249	Gallocatechin	306.29	1.65	0.27
	MOL002258	Physcion-9-O-beta-D-glucopyranoside_qt	300.28	2.48	0.30
	MOL002268	Rhein	284.23	1.88	0.28
	MOL002281	Toralactone	272.27	2.25	0.24
	MOL002286	Laccaic Acid D	300.23	1.61	0.31
	MOL002289	Physcione	284.28	2.15	0.27
	MOL002302	RHAPONTIN	406.42	1.26	0.55
	MOL000471	Aloe-emodin	270.25	1.67	0.24
	MOL000472	Emodin	270.25	2.49	0.24
	MOL000476	Physcion	284.28	2.74	0.27
	MOL000096	(-)-Catechin	290.29	1.92	0.24
Angelica sinensis	MOL005786	Byakangelicin	334.35	1.70	0.35
	MOL005789	Neobyakangelicol	316.33	2.72	0.31
	MOL005790	5-[[(2S)-3,3-dimethyloxiran-2-yl]methoxy]-3,7-dihydropyrano[3,2-f] benzofuran-2-one	288.32	1.47	0.31
	MOL005791	Oxypeucedanin	286.30	2.00	0.30
	MOL005792	{5-[2'(R)-Hydroxy-3'-methyl-3'-butenyl-oxy]furocoumarin}	286.30	2.74	0.26
	MOL005793	9-[[(2R)-3,3-dimethyloxiran-2-yl]methoxy]furo[3,2-g]chromen-7-one	286.30	2.49	0.29
	MOL005800	Byakangelicol	316.33	2.47	0.36
	MOL005806	4-[(2S)-2,3-dihydroxy-3-methylbutoxy]furo[3,2-g]chromen-7-one	304.32	1.72	0.29
	MOL005807	Sen-Byakangelicol	386.43	2.76	0.61
	MOL013430	Prangenin	286.30	2.49	0.29
Ligusticum chuanxiong	MOL001729	Crysophanol	254.25	2.76	0.21
Croton tiglium	MOL001626	Phrymarolin-II	458.45	2.30	0.93
Salvia miltiorrhiza	MOL000569	Digallate	322.24	1.53	0.26
	MOL000006	Luteolin	286.25	2.07	0.25
	MOL006452	1,5-Dihydroxy-3-methylanthraquinone	254.25	2.76	0.21
	MOL007040	1-ketoisocryptotanshinone	310.37	2.59	0.44
	MOL007053	7-oxoroyleanone	330.46	3.00	0.34
	MOL007063	Przewalskin A	398.49	2.25	0.65
	MOL007068	Przewaquinone B	292.30	2.99	0.41
	MOL007070	(6S,7R)-6,7-dihydroxy-1,6-dimethyl-8,9-dihydro-7H-naphtho[8,7-g] benzofuran-10,11-dione	312.34	2.34	0.45
	MOL007071	Przewaquinone F	312.34	2.07	0.46
	MOL007080	Tanshinol A	292.30	2.99	0.41
	MOL007081	Danshenol B	354.48	2.59	0.56
	MOL007082	Danshenol A	336.41	2.01	0.52
	MOL007090	Danshexinkum A	296.34	2.59	0.30
	MOL007093	Danshexinkum D	336.41	2.83	0.55
	MOL007100	Dihydrotanshinlactone	266.31	2.77	0.32
	MOL007101	DihydrotanshinoneI	278.32	2.86	0.36
	MOL007105	Epidanshenspiroketallactone	284.38	2.37	0.31
	MOL007116	Methylrosmarinate	374.37	2.94	0.37
	MOL007120	Miltionone II	312.39	2.14	0.44
	MOL007121	Miltipolone	300.43	2.74	0.37
	MOL007128	Paramiltioic Acid	332.43	2.68	0.37

	MOL007129	Potassium Salvianolate D	418.38	2.33	0.50
	MOL007130	Prolithospermic Acid	314.31	2.77	0.31
	MOL007131	(2S,3S)-2-(3,4-dihydroxyphenyl)-7-hydroxy-4-[(E)-3-hydroxy-3-oxoprop-1-enyl]-2,3-dihydrobenzofuran-3-carboxylic acid	358.32	2.07	0.41
	MOL007132	(2R)-3-(3,4-dihydroxyphenyl)-2-[(Z)-3-(3,4-dihydroxyphenyl)acryloyl]oxy-propionic acid	360.34	2.69	0.35
	MOL007133	Salviacoccin	356.40	1.41	0.63
	MOL007135	Salvianic Acid C	378.36	2.03	0.37
	MOL007138	Salvianolic Acid D	418.38	2.33	0.50
	MOL007140	(Z)-3-[2-[(E)-2-(3,4-dihydroxyphenyl)vinyl]-3,4-dihydroxy-phenyl] acrylic acid	314.31	2.82	0.26
	MOL007141	Salvianolic Acid G	340.30	2.20	0.61
	MOL007143	Salvilenone I	270.40	2.88	0.23
	MOL007150	(6S)-6-hydroxy-1-methyl-6-methylol-8,9-dihydro-7H-naphtho[8,7-g] benzofuran-10,11-quinone	312.34	2.42	0.46
	MOL007151	Tanshindiol B	312.34	2.34	0.45
	MOL007152	Przewaquinone E	312.34	2.34	0.45
	MOL007156	Tanshinone VI	296.34	2.44	0.30
	MOL000008	Apigenin	270.25	2.33	0.21
Aconitum carmichaelii	MOL002384	14-Deoxy-11,12-didehydroandrographolide	332.48	2.10	0.32
	MOL002388	Delphin_qt	303.26	1.67	0.28
	MOL002392	Deltoin	328.39	2.48	0.37
	MOL002398	Karanjin	292.30	2.94	0.34
	MOL002414	Denudatine	343.56	2.25	0.67
	MOL002419	(R)-Norcoclaurine	271.34	2.57	0.21
	MOL002421	Ignavine	449.59	1.22	0.25
	MOL002436	Delavaconitine	497.69	1.38	0.37
Zanthoxylum bungeanum	MOL013271	Kokusaginin	259.28	2.33	0.20
	MOL002663	Skimmianin	259.28	2.33	0.20
	MOL002881	Diosmetin	300.28	2.32	0.27
	MOL000098	Quercetin	302.25	1.50	0.28

Note: MOL ID, molecule identifier; MW, molecular weight; AlogP, adipose-water partition coefficient; DL, drug-likeness.

tively. After eliminating duplicates, 75 unique active ingredients remained (see **Table 5**). Additionally, the drug targets associated with the Dunhuang Mofeng ointment were extracted from the TCMSP database, yielding a total of 908 potential targets. These targets were standardized using the UniProt database, applying the "Human" and "Reviewed" filters, and subsequently converted to standardized gene names. After removing duplicates, a final set of 85 distinct target genes was identified.

3.7 Intersection genes between RA differential genes and Dunhuang Mofeng ointment-related targets

Gene expression datasets associated with RA were obtained from the GEO database, including GSE15573, GSE56649, GSE93272, and GSE97779. The data were preprocessed and normalized using R software, followed by differential gene expression analysis conducted using the limma package. The filtering criteria included |logFC|>1 and adjusted P-value <0.05. Based on these criteria, both upregulated and downregulated differential genes were identified (**Figure 4A**). Specifically, in the GSE15573 dataset, 22 differential genes were identified, of which 21 were upregulated and 1 was downregulated. In the GSE56649 dataset, a total of 724 differential genes were identified, with 409 upregulated and 315 downregulated. The GSE93272 dataset yielded 26 differential genes, 25 of which were upregulated and 1 downregulated. In the GSE97779 dataset, 2792 differential genes were identified, comprising 1240 upregulated genes and 1552 downregulated genes. A heatmap illustrating the expression profiles of these differential genes is shown in **Figure 4B**. After removing duplicates, a total of 3564 unique differential genes were identified, of which 3397 were selected for further analysis (**Figure 4C**). Additionally, intersection analysis between drug targets and RA-related differential genes was performed using R software, revealing 21 intersecting genes: ADRB2, CDKN1A, BAX, TNF, MYC, ACTA2, CCND1, RB1, CDK4, MCL1, COLQ, FOS, RUNX1T1, CCND2, PSMD3, FCER2, TRPM2, STAT1, CXCL11, CXCL10, and SPP1. These intersection genes are depicted in a Venn diagram (**Figure 4D**).

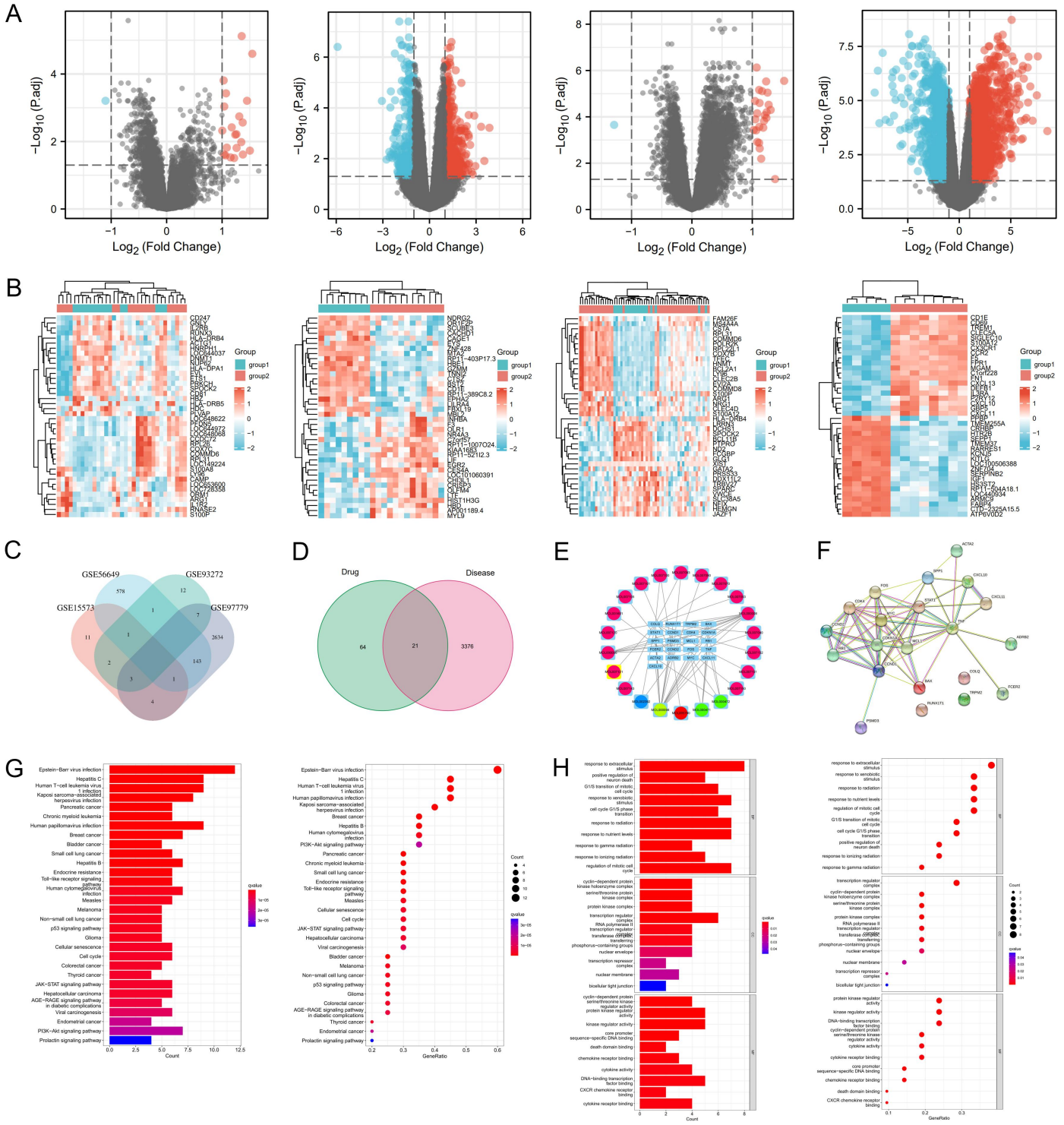

Figure 4. Identification of key targets and pathways of Dunhuang Mofeng ointment in RA based on differential gene and network pharmacology analysis. (A) Volcano plot of differential genes in RA-related gene datasets GSE15573, GSE56649, GSE93272 and GSE97779; (B) Heat maps of differential genes in GSE15573, GSE56649, GSE93272, and GSE97779; (C) Intersection of differential genes across GSE15573, GSE56649, GSE93272, and GSE97779; (D) Intersection genes between Dunhuang Mofeng ointment targets and RA-related genes; (E) Regulatory network of Dunhuang Mofeng ointment active ingredients and their targets; (F) PPI network and core targets of Dunhuang Mofeng ointment in RA treatment; (G) KEGG pathway enrichment analysis; (H) GO functional enrichment analysis. RA, rheumatoid arthritis; PPI, protein-protein interaction; KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology.

Table 6. Core PPI network topology parameters

Name	Betweenness	Closeness	Degree	Eigenvector	LAC	Network
CCND1	44.07	0.773	24	0.345	12.67	18.80
MYC	12.07	0.739	22	0.341	13.82	18.93
CDKN1A	7.41	0.708	20	0.323	14.00	17.57
FOS	9.04	0.708	20	0.322	13.60	16.64
MCL1	3.86	0.680	18	0.302	13.33	15.32
STAT1	16.24	0.680	18	0.277	10.67	13.10
CDK4	5.05	0.680	18	0.304	13.78	15.66

Note: PPI, protein-protein interaction; LAC, local average connectivity.

Table 7. Binding energy of core active ingredients and core protein targets

Protein	PDB ID	Ligand	Grid_size	Affinity (kcal/mol)
CCND1	2w96	Luteolin	66*52*78	-9.0
CCND1	2w96	Apigenin	64*52*76	-8.7
CCND1	2w96	Quercetin	64*52*86	-9.4
MYC	5i4z	Aloe-emodin	98*48*40	-6.7
MYC	5i4z	Emodin	98*48*40	-6.5
MYC	5i4z	Quercetin	98*48*40	-6.3
CDKN1A	6cej	Aloe-emodin	40*40*40	-4.9
CDKN1A	6cej	Emodin	40*40*40	-5.0
CDKN1A	6cej	Luteolin	40*40*40	-5.8
CDKN1A	6cej	Apigenin	40*40*40	-5.4
CDKN1A	6cej	Quercetin	40*40*40	-5.7
MCL1	3mk8	Luteolin	40*40*40	-6.8
MCL1	3mk8	Apigenin	40*40*40	-6.5
STAT1	3wwt	Quercetin	54*40*70	-7.5
FOS	1a02	Apigenin	70*64*84	-8.1
FOS	1a02	Quercetin	76*64*86	-8.1
CDK4	3g33	Luteolin	112*98*94	-8.7
CDK4	3g33	Apigenin	112*96*80	-8.4

Note: PDB ID, Protein Data Bank identifier.

3.8 Construction of the regulatory network diagram for the Dunhuang Mofeng ointment formula

The data files pertaining to the network regulation of TCM compounds, obtained through Perl script execution, were imported into Cytoscape 3.8.0 for further analysis. A "drug - active ingredient - target" regulatory network was constructed (**Figure 4E**). In this network, light blue rectangular nodes represent the targets, while circular nodes denote the active ingredients of TCM. The edges connecting the nodes indicate that an active ingredient can modulate the corresponding RA-related target, with the line density reflecting the strength of the interaction. The top five active ingredients, ranked by the number of interactions, are as follows: MOL000098 (quercetin), interacting with 15 targets; MOL000008 (apigenin), interacting with 13 targets; MOL000006 (luteolin), interacting with 6 targets; MOL000471 (aloe-emodin), interacting with 4 targets; and MOL000472 (emodin), also interacting with 4 targets. Each active ingredient exhibits both independent regulatory effects and shared targets.

3.9 PPI network and core target analysis of Dunhuang Mofeng ointment in treating RA

The 21 "drug-disease" common targets were input into the STRING database to retrieve protein interaction data, and the resulting "string_interactions.tsv" file was downloaded. This file was subsequently imported into Cytoscape software, where the CytoNCA plugin was employed to calculate the network parameters of each protein, generating the scoring file "score1.csv". The R script was used to filter the "score1" file based on the following parameters: betweenness =2.63, closeness =0.60, degree =13, eigenvector =0.23, local average connectivity =9.71, and network =11.75. By applying these criteria, the core target proteins were identified as CCND1, MYC, CDKN1A, FOS, MCL1, STAT1, and CDK4. The topological parameters of these core proteins are ranked according to the degree score and presented in **Table 6** and **Figure 4F**.

3.10 GO enrichment and KEGG pathway analysis

GO enrichment analysis was performed using R software, yielding 1036 GO terms. The top 10 terms in each GO category (biological process, cellular component, and molecular function) were selected and visualized as bar and bubble charts (**Figure 4H**). Biological process enrichment showed thattargets were primarily involved in response to extracellular stimulus, G1/S transition of mitotic cell cycle, response to radiation, and regulation of the mitotic cell cycle. Cellular component enrichment indicated that target proteins were mainly localized in the cyclin-dependent protein kinase holoenzyme complex, serine/threonine protein kinase complex, transcription regulator complex, nuclear envelope, and bicellular tight junction. Molecular function enrichment revealed that targets was primarily associated with cyclin-dependent protein serine/threonine kinase regulator activity, protein kinase regulator activity, cytokine activity, and chemokine receptor binding. KEGG pathway enrichment analysis was performed using R software, identifying 90 signaling pathways. The top 30 significantly enriched pathways were selected and visualized as a bubble plot (**Figure 4G**). The results showed that the target was mainly enriched in the AGE-RAGE, JAK-STAT, PI3K-Akt, Toll-like receptor, and p53 signaling pathways, as well as pathways related to cellular senescence, cell cycle, cancer, viral infection, and diabetic complications.

3.11 Molecular docking

Molecular docking of small molecule ligands with protein receptors was performed using AutoDock Vina, and the docking results were evaluated based on affinity values (**Table 7**).

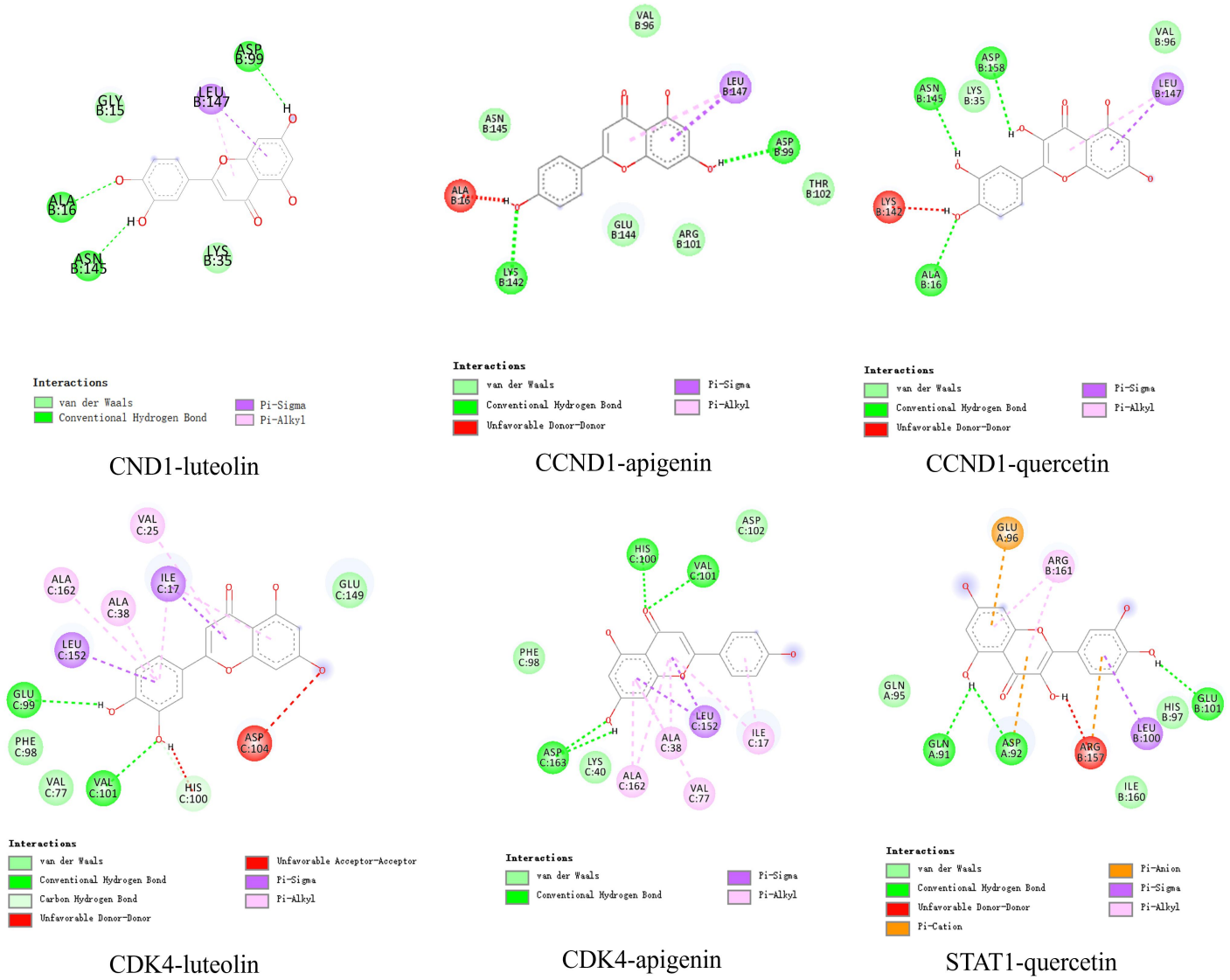

Figure 5. Molecular docking analysis of key targets and active compounds of Dunhuang Mofeng ointment.

The molecular docking results showed that the active compounds formed stable interaction networks with multiple target proteins within the binding pockets. Specifically, the ligands were able to form stable interactions with key amino acid residues (such as ASP, ASN, LYS, GLU, and HIS) through conventional hydrogen bonds, while also engaging in various non-covalent interactions including π–sigma, π–alkyl, and van der Waals interactions, which further enhanced the stability of ligand–protein binding. In addition, unfavorable donor–donor interactions and other weak interactions were observed in some complexes, suggesting a certain degree of conformational adaptability of the ligands within the binding sites. Overall, these multiple intermolecular interactions collectively contribute to maintaining the stable binding of the ligands within the active pockets of the target proteins, providing a structural basis for their potential biological activities (**Figure 5**).

3.12 mRNA expression levels of STAT1, CDK4, CCND1, MYC, CDKN1A, MCL1, and FOS in ankle joints of rats from different experimental groups

Compared to the control group, the mRNA expression levels of STAT1, CDK4, CCND1, MCL1, FOS, and MYC in the ankle joints of rats in the model group were significantly elevated ($P<0.001$), whereas the mRNA expression of CDKN1A was significantly reduced ($P<0.001$). In contrast, rats in all drug-treated groups exhibited significantly lower mRNA expressions of STAT1, CDK4, CCND1, MCL1, FOS, and MYC ($P<0.05$) compared to the model group, while CDKN1A mRNA expression was markedly increased ($P<0.001$). Notably, the medium-dose and Western medicine groups showed the most pronounced effects (**Figure 6**).

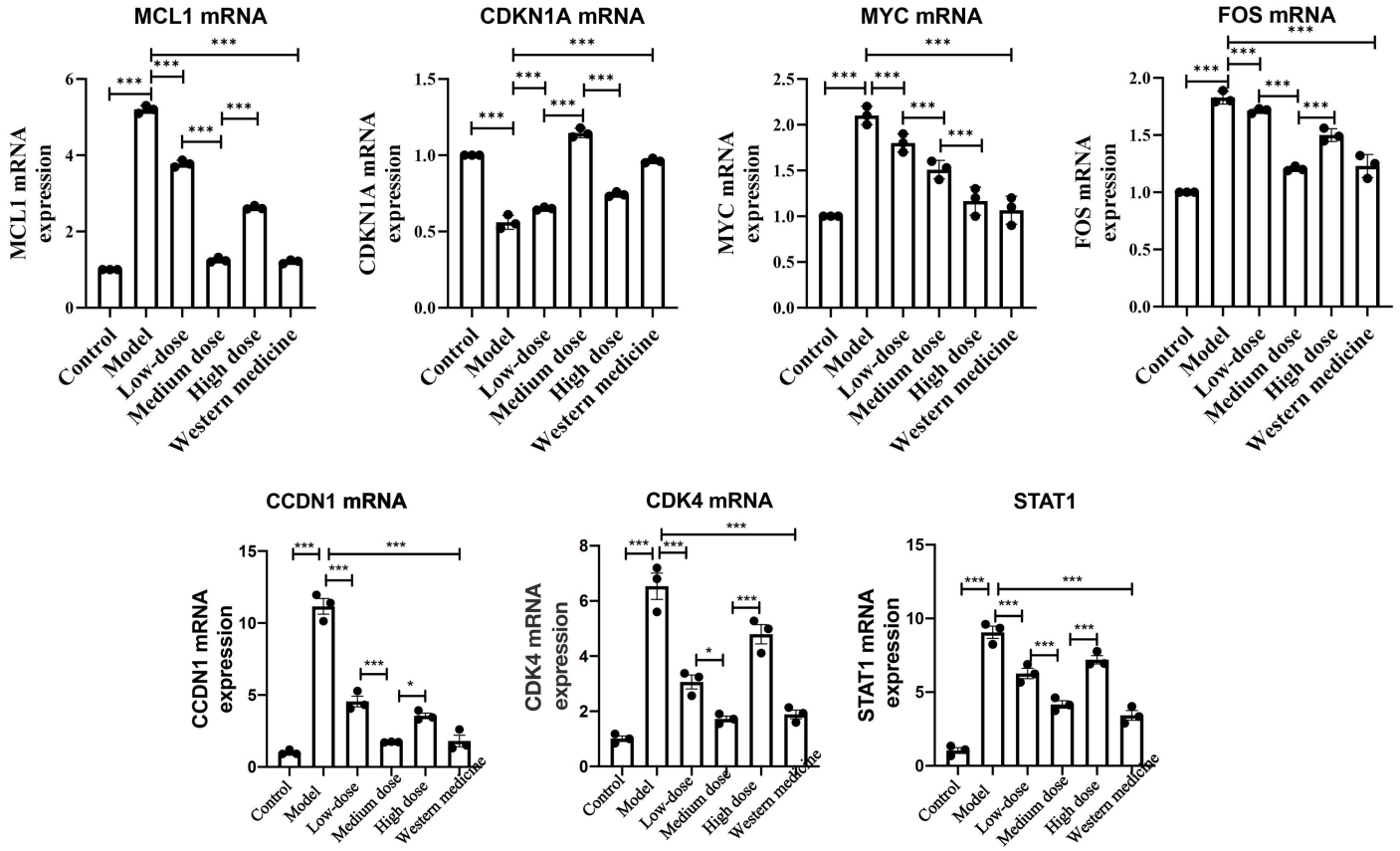

Figure 6. mRNA expression levels of target genes in the right ankle joint of rats in each group. *P<0.05, **P<0.01, ***P<0.001.

4 DISCUSSION

This study elucidates the potential mechanisms underlying the therapeutic effects of Dunhuang Mofeng ointment in the intervention of RA through network pharmacological analysis. The findings identify quercetin, apigenin, luteolin, aloe-emodin, and emodin as the key active ingredients of the formulation. RA, a chronic autoimmune disease, is characterized by joint inflammation, progressive damage, and, in severe cases, disability or mortality. Among these compounds, quercetin, a natural flavonoid with diverse biological activities, plays a pivotal role in RA management. Quercetin demonstrates pronounced anti-inflammatory and protective effects through multiple mechanisms. Studies indicate that quercetin can reverse neurodegenerative processes in the enteric nervous system and exhibit cytoprotective, genoprotective, and hepatoprotective properties [11]. In the context of RA, quercetin ameliorates inflammatory responses by modulating neutrophil activity, including inhibiting neutrophil infiltration, reducing plasma inflammatory cytokine levels, and promoting the apoptosis of activated neutrophils. Furthermore, it decreases the formation of neutrophil extracellular traps by suppressing autophagy, positioning quercetin as a promising candidate for RA treatment [12]. Clinical trials support these findings, revealing that an 8-week supplementation with quercetin significantly alleviated morning stiffness, morning pain, and post-activity pain in RA patients ($p<0.05$). Disease Activity Score and Health Assessment Questionnaire scores were markedly lower in the quercetin group compared to the placebo group, with a notable reduction in the proportion of patients exhibiting active disease. Additionally, plasma levels of high-sensitivity TNF-α were significantly reduced ($p<0.05$). Although no significant effect on erythrocyte sedimentation rate was observed ($p>0.05$), the number of tender joints was significantly decreased [13]. Mechanistic studies further highlight quercetin's multi-target regulatory potential. Quercetin mitigates IL-17-mediated activation of mTOR, extracellular signal-regulated kinase (ERK), and IκB-α pathways, thereby inhibiting osteoclastogenesis in RA. It also reduces RANKL expression in IL-17-induced RA synovial fibroblasts (RA-FLS), attenuating osteoclast differentiation. Moreover, quercetin regulates inflammatory responses in RA by inhibiting Th17 cell differentiation and IL-17 production, while sparing regulatory T cells [14]. In conclusion, quercetin, as a principal active ingredient of Dunhuang Mofeng ointment, exhibits a multi-target, multi-mechanism mode of action, offering a robust theoretical foundation and novel directions for the development of therapeutic agents for RA.

Apigenin, a natural flavonoid with chemopreventive properties, demonstrates significant pro-apoptotic activity across various

cell types. Studies have revealed that apigenin markedly inhibits TRAIL-induced proliferation of RA-FLS while restoring the expression of cell cycle inhibitors p21 and p27 [15]. This inhibitory effect is mediated through disruption of TRAIL-induced activation of the PI3K/Akt signaling pathway. Additionally, apigenin treatment triggers activation of MAPK ERK1/2, with pre-treatment using the ERK inhibitor PD98059 significantly attenuating apigenin-induced apoptosis. Apigenin-induced ERK1/2 activation and apoptosis are closely associated with the generation of intracellular ROS, a process that can be abrogated by the antioxidant Tiron, which blocks ROS production, ERK1/2 phosphorylation, and apoptotic cell death [16]. High-performance liquid chromatography analysis confirmed that the PEG-MSN carrier prolongs the half-life and enhances the stability of luteolin in vivo. Both luteolin and its nanocomplexes significantly suppress the expression of inflammatory markers, including TNF-α, IL-6, IL-1β, and IL-17. Histopathological evaluations corroborate these findings, showing reduced inflammatory cell infiltration, synovial hyperplasia, and joint destruction. Furthermore, luteolin inhibits the expression of key inflammatory targets, including P2X4, NLRP1, ASC, and Caspase-1 p10, highlighting its potent anti-inflammatory and joint-protective properties [17]. In conclusion, apigenin and luteolin exhibit substantial anti-RA potential via multi-target and multi-pathway modulation of inflammation and apoptosis. Their pharmacokinetic profiles can be further optimized through nanocarrier technologies, offering robust theoretical support and promising candidates for the prevention and treatment of RA.

Aloe-emodin effectively suppresses the production of nitric oxide, IL-6, and IL-1β in RAW264.7 cells stimulated by LPS, without inducing significant cytotoxic effects. Concurrently, the mRNA expression levels of iNOS, IL-6, and IL-1β were markedly downregulated. Western blot analysis further demonstrated that aloe-emodin attenuated LPS-induced iNOS protein expression, IκBα degradation, and the phosphorylation of ERK, p38, JNK, and Akt [18]. Emodin, another active compound, exhibits bidirectional immunomodulatory effects in RA by modulating the secretion of both pro-inflammatory cytokines (e.g., TNF-α, IL-6, IL-1β, and IL-17) and anti-inflammatory cytokines (e.g., IL-4, IL-10, and TGF-β), thereby inhibiting inflammation while promoting tissue repair [19]. Furthermore, emodin alleviates inflammatory arthritis, such as adjuvant-induced arthritis, by reducing neutrophil infiltration, promoting neutrophil apoptosis, and inhibiting autophagy and the formation of neutrophil extracellular traps [20]. These findings underscore the significant anti-inflammatory and anti-RA potential of emodin.

Molecular docking studies revealed that the active components of Dunhuang Mofeng extract, including aloe-emodin, exhibit strong binding affinities (binding energy ≤-5 kcal/mol) to key RA-related targets, such as CCND1, MYC, CDKN1A, FOS, MCL1, STAT1, and CDK4. Notably, the binding energy for CDKN1A was calculated at -4.9 kcal/mol. Overexpression of CCND1 and CDK4, coupled with the downregulation of p27, constitutes a pivotal molecular event in RA pathogenesis [21]. Additionally, CIP2A has been identified as a promoter of RA progression by enhancing synovial cell resistance to apoptosis, independent of c-Myc expression [22]. Under acidic conditions, activation of ASIC1a drives RA synovial cell proliferation via the Wnt/β-catenin/c-Myc signaling pathway, an effect significantly attenuated by the ASIC1a inhibitor Psalmotoxin-1 (PcTx-1). In vivo studies corroborate that PcTx-1 mitigates synovial hyperplasia and cartilage degradation in adjuvant arthritis models [23]. In RA synovial tissue and fibroblast-like synovial cells (HFLS-RA), CDKN1A expression is significantly downregulated. Overexpression of CDKN1A induces G0/G1 cell cycle arrest, thereby inhibiting HFLS-RA proliferation and invasion, reducing pro-inflammatory cytokine levels (TNF-α and IL-6), and increasing anti-inflammatory cytokine expression (IL-10) [24]. Elevated miR-146a levels in RA tissues negatively regulate CDKN1A, while anti-miR-146a intervention effectively reduces HFLS-RA proliferation and invasion, decreases pro-inflammatory cytokine expression, and enhances anti-inflammatory cytokine levels [24]. Curcumin, another RA therapeutic candidate, has been shown to inhibit osteoclastogenesis in peripheral blood mononuclear cells by suppressing MAPK signaling pathways, including ERK1/2, p38, and JNK. This suppression inhibits the expression of RANK, c-Fos, and NFATc1, further highlighting its therapeutic potential [25]. Collectively, these findings suggest that the core components of Dunhuang Mofeng ointment exert multi-target therapeutic effects in RA by modulating key inflammatory and cellular signaling pathways. This multi-faceted approach underscores the formulation's potential as an effective intervention for RA.

MCL1, a pivotal anti-apoptotic molecule within the Bcl-2 family, plays an indispensable role in the survival of T and B lymphocytes as well as macrophages. In the synovial fluid of RA patients, CD14+ macrophages exhibit markedly elevated MCL1 expression. Inhibition of the PI3K/Akt-1 or STAT3 signaling pathways significantly reduces the proportion of CD14+ macrophages in synovial fluid, concomitantly downregulating MCL1 expression and inducing apoptosis in synovial macrophages. Furthermore, transfection of RA synovial macrophages with MCL1-specific siRNA induces apoptotic cell death, underscoring the critical role of MCL1 in macrophage survival [26]. Within the synovial tissue of RA patients, MCL1 expression is significantly upregulated in both the synovial membrane and subsynovial fibroblasts compared to control groups. This heightened expression correlates positively with inflammation severity and TNF-α levels. In vitro studies demonstrate that IL-1β stimulation induces a substantial increase in MCL1 mRNA and protein expression in synovial fibroblasts, a process essential for fibroblast survival. Adenovirus-mediated antisense MCL1 expression triggers fibroblast apoptosis via activation of pro-apoptotic molecules Bax, Bak, and Bim [27]. Elevated total STAT1 protein levels, alongside its activated

forms (tyrosine and serine phosphorylated), are consistently observed in RA synovium. STAT1 is predominantly localized in T and B lymphocytes undergoing focal inflammatory infiltration and in endosynovial fibroblast-like cells [28]. In vitro, fibroblast-like synoviocytes derived from RA patients exhibit constitutively higher STAT1 levels compared to those from osteoarthritis patients, with phosphorylation rapidly induced following IFN-β stimulation. Moreover, clinical studies have demonstrated that the efficacy of Baricitinib in RA patients correlates strongly with STAT1 phosphorylation in monocytes, suggesting that STAT1 phosphorylation may serve as an early biomarker for therapeutic response and a valuable reference point for treatment optimization [29]. CDK4/6, critical regulators of the cell cycle, also emerge as significant contributors to RA pathology. A double-blind, placebo-controlled Phase 1b clinical trial of the CDK4/6 inhibitor CTK-276 (≤175 mg) reported favorable tolerability in patients with active RA, with no significant safety concerns. At doses of ≥25 mg/day, TCK-276 demonstrated preliminary clinical efficacy, offering a promising avenue for further investigation into its therapeutic potential in active RA [30].

Although the classical TCMSP screening parameters (MW≤ 500, AlogP 1-3, and DL≥0.18) were originally developed for evaluating the DL of orally administered compounds, MW and lipophilicity are also key determinants influencing skin permeability. In general, compounds with MWs below 500 Da and moderate lipophilicity are considered more favorable for transdermal penetration. However, additional physicochemical factors, including the skin permeability coefficient, hydrogen bonding capacity, and polar surface area, may also influence the efficiency of transdermal delivery. Meanwhile, regardless of whether drugs are administered orally or topically, their absorption and distribution processes in vivo are affected by multiple physicochemical and biological factors. Therefore, DL parameters based on physicochemical properties may still provide certain reference value. In addition, the network pharmacology results in this study were further supported by mRNA expression analysis and animal experimental evidence, suggesting that the potential active components identified based on the above screening criteria are reliable within the context of this study. Therefore, the screening strategy adopted in this study primarily aims to identify potential bioactive compounds with reasonable physicochemical properties rather than to directly predict their transdermal pharmacokinetic characteristics. Regarding the limitations of this study, the validation of the key signaling pathways predicted by network pharmacology was mainly performed at the mRNA level, while the corresponding protein expression levels and phosphorylation status were not further examined. Due to factors such as post-transcriptional regulation, changes in mRNA expression do not necessarily fully reflect protein expression levels. Therefore, future studies are still required to further verify the related signaling pathways at the protein level using methods such as Western blot or other protein-based assays. On the other hand, the present study has already provided experimental validation of the predicted results at the animal level. Compared with studies that only perform validation at the cellular level, the in vivo validation conducted in this study enhances the reliability and biological relevance of the findings to a certain extent.

5 CONCLUSION

Through network pharmacological analysis and molecular docking studies, this investigation identified the core active constituents of Dunhuang Mofeng extract, namely quercetin, apigenin, luteolin, aloe-emodin, and emodin, with key molecular targets including CCND1, MYC, CDKN1A, FOS, MCL1, STAT1, and CDK4. In vitro pharmacodynamic assays further substantiated the significant therapeutic effects of Dunhuang Mofeng ointment in CIA rats. The underlying mechanism of action appears to involve the inhibition of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, while simultaneously promoting the release of the anti-inflammatory cytokine IL-10. These actions collectively contribute to the alleviation of foot and ankle joint swelling and pain, as well as the reduction of tissue edema in the rats. Additionally, the validation of molecular docking predictions was achieved by comparing the expression levels of STAT1, CDK4, CCND1, MCL1, FOS, CDKN1A, and MYC across experimental groups, confirming that the therapeutic effects of Dunhuang Mofeng ointment are likely mediated through the downregulation of these critical mRNA markers. This study provides a robust theoretical foundation for the continued investigation of Dunhuang Mofeng ointment as a potential treatment for RA. However, despite the successful achievement of the study objectives, there are inherent limitations in the experimental design, particularly concerning the selection of experimental endpoints and the need for further validation. Future research should focus on expanding the range of experimental indicators to facilitate a more comprehensive and in-depth elucidation of the precise molecular mechanisms underlying the observed therapeutic effects.

DECLARATIONS

Author contributions

HlZ conceived the initial idea of the study, collected and organized the relevant literature and data, performed data processing and preliminary analysis, prepared the figures and tables, and drafted the manuscript. JP assisted with data collection, literature screening, data curation, and manuscript formatting, and participated in revising the draft for important intellectual content. ZJS supervised the overall study, contributed to the study design and framework development, critically revised the manuscript for important intellectual content, and approved the final version for submission.

Funding

The authors declare that financial support was received for the research, authorship, and/or publication of this article. This

study was supported by the National Natural Science Foundation of China (81960877), the University Innovation Fund of Gansu Province (2021A-076), the Gansu Province Science and Technology Plan (Innovation Base and Talent Plan) (21JR7RA561), the Natural Science Foundation of Gansu Province (21JR1RA267), the Education Technology Innovation Project of Gansu Province (2022A-067), and the Open Project of the Key Laboratory of Dunhuang Medicine and Transformation, Ministry of Education (DHYX21-07, DHYX22-05, and DHYX21-01). Additional support was provided by the Gansu Province Joint Fund General Program (24JRRA878), the Gansu Provincial Science and Technology Program Project (24JRRA1020 and 25JRRA249), the Gansu Province Key Talent Program (2025RCXM006), the Teaching Research and Reform Program for Postgraduate Education at Gansu University of Traditional Chinese Medicine (YBXM-202406), and the Special Fund for Mentors of "Qihuang Talents" in the First-Level Discipline of Chinese Medicine (ZYXKBD-202415 and ZYXKSD-202415).

Data availability

The datasets used or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

All animal experiments in this study were conducted in accordance with the relevant guidelines and regulations and were approved by the Animal Ethics Committee of Gansu University of Chinese Medicine (Approval Number: SY2023-966).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

Not applicable.

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