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# A novel nasopharyngeal airway set-Nasal Pharyngeal Set-flexible

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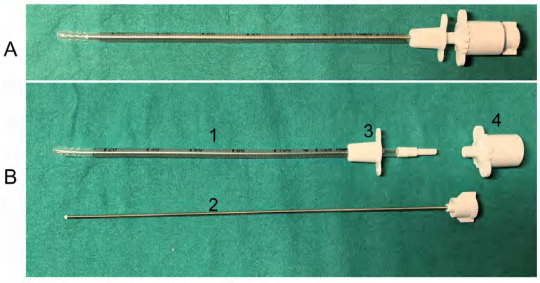
Many patients, such as those who are obese, are prone to hypoxia during anaesthesia induction, painless gastroenteroscopy, and ICU sedation, which can lead to severe organ damage [1]. The traditional nasopharyngeal airway can effectively alleviate this problem, but it also introduces complications such as epistaxis [2]. NaPS$^{flex}$ (Nasal Pharyngeal Set-flexible) is a novel nasopharyngeal airway set developed by our team, designed to address difficult airways safely and simply. It has been registered as a medical device in China (Zhejiang Medical Device Registration Approval No. 20232081212).

NaPS$^{flex}$ consists of a reinforced catheter, a spring core, a restrictive nose plug, and an adapter (**Figure 1**). The reinforced catheter has small holes underneath for ventilation. With an outer diameter of only 4 mm, it prevents nasopharyngeal compression damage during catheter retention. The catheter is made of a flexible material to reduce the risk of nasal mucosa abrasion. The spring core is flexible, allowing easy access to the nasopharyngeal lumen. The restrictive nose plug regulates the catheter's depth into the nasal cavity and helps secure it in place. Additionally, the catheter can be connected to either a nasal cannula or an anaesthesia machine's breathing line via the adapter for oxygenation.

Several randomized controlled studies, approved by the Ethics Committee of the Naval Medical University and currently in the process of data collection, may provide robust evidence of its safety and efficacy. We believe that the new nasopharyngeal airway set can be widely adopted by anesthesiologists due to its potential in airway management.




1: reinforced catheter; 2: spring core
3: restrictive nose plug; 4: adapter

**Figure 1. NaPS$^{flex}$ structure.** (A) NaPS$^{flex}$; (B) Components of NaPS$^{flex}$.

**Author contributions:** Zui Zou conceived the original idea and supervised the project. Chenglong Zhu wrote the manuscript. Wenyun Xu helped shape the manuscript. All authors contributed to the article.

## References

[1] Wang C, Zhao N, Wang W, et al. Intraoperative mechanical ventilation strategies for obese patients: a systematic review and network meta-analysis. Obes Rev 2015;16(6):508-517.

[2] Mort TC. Complications of emergency tracheal intubation: immediate airway-related consequences: part II. J Intensive Care Med 2007;22(4):208-215.

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# Rescue of rocuronium-induced refractory anaphylaxis with sugammadex in a laparoscopic hysterectomy: A case report

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## Highlights

- Anaphylaxis is a potentially life-threatening event during general anesthesia, with a rare incidence estimated at 1 in 10,000 to 1 in 20,000 cases.
- Neuromuscular blocking agents, antibiotics, and latex are the primary triggers of perioperative anaphylaxis, with rocuronium being one of the most commonly implicated drugs.
- Sugammadex is a valuable drug for reversing rocuronium-induced refractory anaphylaxis.

## Abstract

Neuromuscular blocking agents, particularly rocuronium, are a major cause of perioperative anaphylaxis. This report presents a rare and severe case of rocuronium-induced anaphylaxis in a 50-year-old woman undergoing an elective total laparoscopic hysterectomy at a tertiary care center in Eastern Nepal. Despite multiple doses of intravenous epinephrine, the patient did not achieve adequate hemodynamic recovery. However, a single bolus of sugammadex resulted in the rapid and complete resolution of symptoms. This case highlights the potential of sugammadex as a life-saving adjunct in treating neuromuscular blocking agents-induced anaphylaxis that is unresponsive to standard interventions.

**Keywords:** Rocuronium, sugammadex, anaphylaxis, epinephrine, refractory

## Introduction

Anaphylaxis is a potentially life-threatening event following general anesthesia, with a rare incidence estimated to be between 1 in 10,000 and 1 in 20,000 cases [1]. Neuromuscular blocking agents (NMBAs), antibiotics, and latex account for the majority of perioperative anaphylactic reactions, with rocuronium being one of the most frequently involved agents [2]. NMBA-induced anaphylaxis develops rapidly, typically within minutes after drug administration, and manifests as cardiovascular collapse, bronchospasm, urticaria, or angioedema [3]. Hypersensitivity reactions to rocuronium, a non-depolarizing aminosteroidal NMBA, occur due to the presence of quaternary ammoniums, which are strong allergens [4]. Standard treatment for anaphylaxis focuses on immediate epinephrine administration (intramuscular or intravenous), fluid replacement, corticosteroids, and antihistamines [5].

However, not all cases respond to first-line therapy. Sugammadex, a modified form of gamma-cyclodextrin, has been investigated as a reversal agent for aminosteroidal neuromuscular blockade, with recent interest in its application for NMBA-induced anaphylaxis [6]. By binding free circulating rocuronium molecules, sugammadex may accelerate the resolution of symptoms [7]. This case presents a rare instance

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**Table 1. Timeline of Events and Vital Sign Changes**

| Time (min) | MAP (mmHg) | $SpO_2$ (%) | HR (bpm) |
|---|---|---|---|
| 0 (Baseline) | 85 | 98 | 75 |
| 2 (Induction) | 80 | 97 | 89 |
| 5 (5 minutes after rocuronium administration) | 63 | 94 | 116 |
| 10 (10 minutes after rocuronium administration) | 50 | 86 | 133 |
| 15 (15 minutes after rocuronium administration indicating severe reaction) | 40 | 80 | 142 |
| 20 (Post-sugammadex) | 60 | 90 | 110 |

Note: MAP, mean arterial pressure; $SpO_2$, peripheral capillary oxygen saturation; HR, heart rate.

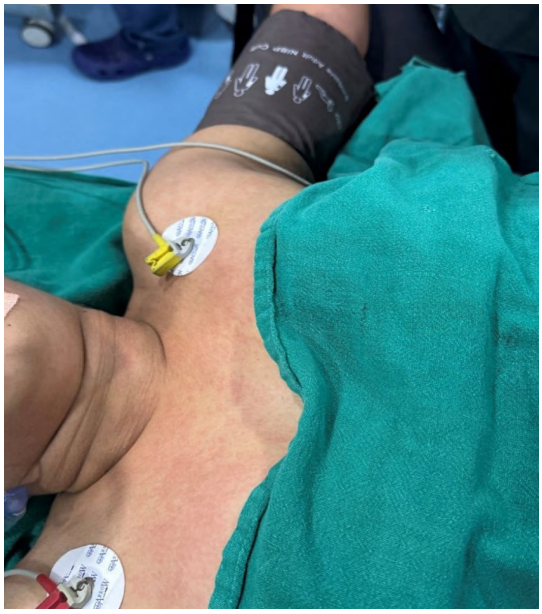

**Figure 1. Erythematous rashes over the chest and arm.**

of rocuronium-induced anaphylaxis that was unresponsive to epinephrine but successfully reversed with sugammadex.

## Case presentation

A 50-year-old woman weighing 65 kg with ASA physical status, I underwent an elective total laparoscopic hysterectomy for abnormal uterine bleeding. She had no significant medical history, drug allergies, or prior surgeries. Laboratory data, including complete blood count, renal and hepatic function, electrolytes, ECG, and chest X-ray, were all within normal limits.

Upon entering the operating room, standard ASA monitoring was initiated. Pre-anesthetic medications included intravenous glycopyrrolate (0.2 mg), midazolam (1 mg), and fentanyl (100 μg). The patient received intravenous propofol (120 mg) and rocuronium (50 mg). Shortly after rocuronium administration, erythematous rashes appeared on the chest and neck, accompanied by hypotension (mean arterial pressure [MAP] 63 mmHg), tachycardia (heart rate [HR] 116 bpm), and oxygen desaturation ($SpO_2$ 94%) (**Figure 1**). Within one to two minutes, her condition worsened significantly, with MAP decreasing to 40 mmHg, HR increasing to 142 bpm, and $SpO_2$ dropping to 80%. These signs were consistent with severe anaphylaxis.

Despite timely intravenous fluid resuscitation and a total of 300 μg epinephrine, the patient's hemodynamic instability and hypoxia persisted, rendering the anaphylactic reaction refractory. Given the temporal relationship with rocuronium administration and the failure of conventional therapy, sugammadex was considered as a potential rescue therapy.

Treatment: At 15 minutes post-induction, 200 mg intravenous sugammadex was administered. Within three minutes, her MAP increased to 60 mmHg, $SpO_2$ improved to 95%, and HR decreased to 110 bpm. Simultaneously, the erythematous rashes and respiratory symptoms rapidly resolved rapidly. Additional treatments, including 100 mg intravenous hydrocortisone and 10 mg chlorpheniramine, were administered to further manage the reaction. The timeline of events and changes in vital signs are presented in **Table 1**.

Due to the severity of the reaction, the surgery was postponed. The patient was transferred to the intensive care unit for close monitoring and did not experience any further complications. She was discharged in good condition on the third postoperative day. A skin prick test six weeks later confirmed rocuronium as the causative agent.

## Discussion

This case presents a rare and severe episode of rocuronium-induced anaphylaxis, which was refractory to conventional epinephrine treatment. It highlights the challenges in managing severe hypersensitivity reactions and underscores the emerging role of sugammadex as a potential rescue agent.

Perioperative anaphylaxis is a critical emergency, with NMBAs being the most frequent cause, responsible for 50%-70% of reactions [8]. Rocuronium, a steroidal NMBA, is particularly im-

plicated due to its quaternary ammonium ion, which can trigger IgE-mediated cross-linking on mast cells and basophils in sensitized individuals [4, 9]. The acute onset of hypotension, tachycardia, hypoxia, and erythema observed in our patient immediately following rocuronium administration is characteristic of an IgE-mediated anaphylactic response.

While epinephrine remains the cornerstone of anaphylaxis management, its efficacy can be limited if the inciting antigen remains in circulation, perpetuating mast cell and basophil degranulation [10]. This likely explains the refractory hypotension in our patient despite multiple epinephrine boluses.

Sugammadex, a modified gamma-cyclodextrin, is designed to encapsulate steroidal NMBAs like rocuronium in a tight 1:1 complex, rapidly reducing the free plasma concentrations of the drug [6, 11]. Beyond its primary role in reversing neuromuscular blockade, sugammadex's mechanism of action offers a rational basis for its use in treating rocuronium-induced anaphylaxis by eliminating the triggering antigen [12]. The immediate and dramatic improvement in hemodynamics and respiratory function following sugammadex administration in this case supports its potential as a life-saving therapy. Emerging evidence suggests that sugammadex may also stabilize mast cells by preventing further cross-linking of IgE antibodies, thereby attenuating ongoing degranulation [13, 14].

However, sugammadex is highly specific. It is effective only in cases of anaphylaxis induced by steroidal NMBAs such as rocuronium and vecuronium, and is not useful for treating reactions caused by non-steroidal NMBAs (e.g., succinylcholine), antibiotics, latex, or other agents. Additionally, clinicians must be aware of potential drug interactions, such as its ability to bind to steroidal contraceptives and other medications like torcemide, necessitating alternative non-hormonal contraceptive methods for a period following sugammadex administration. While sugammadex's effect on coagulation is minimal, a transient increase in activated partial thromboplastin time and prothrombin time has been observed, although there is no clinical evidence of an increased bleeding risk [11].

The potential for adverse drug reactions to sugammadex itself must also be considered. Although rare, hypersensitivity reactions, including anaphylaxis, have been reported [15]. Common adverse effects include altered taste sensation, nausea, vomiting, headache, bradycardia and QTc prolongation. These are generally managed with symptomatic treatment, such as antiemetics for nausea and vomiting, analgesics for headache, atropine or glycopyrollate for bradycardia, and vigilalant hemodynamic monitoring for arrhythmias and QTc prolongation [11]. Thus, its use should be reserved for clearly refractory cases in which rocuronium-induced anaphylaxis is strongly suspected. Sugammadex is not a substitute for first-line anaphylaxis management with epinephrine, fluids, corticosteroids, and antihistamines, but rather serves as an adjunctive therapy when these measures fail.

This case is particularly relevant in resource-limited settings where advanced diagnostics like serum tryptase assays may not be available. In such situations, clinical acumen and a high index of suspicion are crucial. The empirical use of sugammadex in a refractory anaphylactic scenario temporally linked to rocuronium administration can be life-saving.

**Author contributions:** Conceptualization: Rahul Kumar Chaudhary, Prajwal Bista, Lalit Kumar Rajbanshi, Kanak Khanal. Writing–original draft: Rahul Kumar Chaudhary. Writing–review & editing: Rahul Kumar Chaudhary.

## References

[1] Mertes PM, Malinovsky JM, Jouffroy L, et al. Reducing the risk of anaphylaxis during anesthesia: 2011 updated guidelines for clinical practice. J Investig Allergol Clin Immunol 2011;21(6):442-453.

[2] Harper NJN, Cook TM, Garcez T, et al. Anaesthesia, surgery, and life-threatening allergic reactions: epidemiology and clinical features of perioperative anaphylaxis in the 6th National Audit Project (NAP6). Br J Anaesth 2018;121(1):159-171.

[3] Ebo DG, Clarke RC, Mertes PM, et al. Molecular mechanisms and pathophysiology of perioperative hypersensitivity and anaphylaxis: a narrative review. Br J Anaesth 2019;123(1):e38-e49.

[4] Tuba Z, Maho S, Vizi ES. Synthesis and structure-activity relationships of neuromuscular blocking agents. Curr Med Chem 2002;9:1507–36.

[5] Simons FE, Ardusso LR, Bilò MB, et al. World allergy organization guidelines for the assessment and management of anaphylaxis. World Allergy Organ J 2011;4(2):13-37.

[6] Bom A, Bradley M, Cameron K, et al. A novel concept of reversing neuromuscular block: chemical encapsulation of rocuronium bromide by a cyclodextrin-based synthetic host. Angew Chem Int Ed Engl 2002;41(2):266-

270.
[7] McDonnell NJ, Pavy TJ, Green LK, et al. Sugammadex in the management of rocuronium-induced anaphylaxis. Br J Anaesth 2011;106(2):199-201.
[8] Sadleir PH, Clarke RC, Bunning DL, et al. Anaphylaxis to neuromuscular blocking drugs: incidence and cross-reactivity in Western Australia from 2002 to 2011. Br J Anaesth 2013;110(6):981-987.
[9] Garvey LH, Ebo DG, Mertes PM, et al. An EAACI position paper on the investigation of perioperative immediate hypersensitivity reactions. Allergy 2019;74(10):1872-1884.
[10] Lieberman P, Nicklas RA, Randolph C, et al. Anaphylaxis-a practice parameter update 2015. Ann Allergy Asthma Immunol 2015;115(5):341-384.
[11] Peeters PAM, van den Heuvel MW, van Heumen E, et al. Safety, tolerability and pharmacokinetics of sugammadex using single high doses (up to 96 mg/kg) in healthy adult subjects: a randomized, double-blind, crossover, placebo-controlled, single-centre study. Clin Drug Investig 2010;30:867–74.
[12] Menéndez-Ozcoidi L, Ortiz-Gómez JR, Olaguibel-Ribero JM, et al. Allergy to low dose sugammadex. Anaesthesia 2011;66:217–9.
[13] Clarke RC, Sadleir PH, Platt PR. The role of sugammadex in the development and modification of an allergic response to rocuronium: evidence from a cutaneous model. Anaesthesia 2012;67(3):266-273.
[14] Muraro A, Roberts G, Worm M, et al. Anaphylaxis: guidelines from the European Academy of Allergy and Clinical Immunology. Allergy 2014;69(8):1026-1045.
[15] Takazawa T, Mitsuhata H, Mertes PM. Sugammadex and rocuronium-induced anaphylaxis. J Anesth 2016;30(2):290-297.

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Review Article https://doi.org/10.61189/949147feziwr

# Bibliometric analysis of traditional Chinese medicine in the prevention and treatment of Alzheimer's disease (2015-2024)

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## Highlights

- Summarize the latest research advances in Alzheimer's disease, including its pathogenesis, physiological biomarkers, and therapeutic strategies.
- Analyze the publication trends and current landscape of research on traditional Chinese medicine for Alzheimer's disease prevention and treatment using both quantitative and qualitative bibliometric methods.
- Summarize the therapeutic effects of traditional Chinese medicine and the discovery of novel treatment targets and pathways.
- Develop novel perioperative neurocognitive disorder therapies from Alzheimer's TCM research.

## Abstract

**Objective**: Alzheimer's disease (AD), the most common form of dementia, poses a significant global public health challenge. This study aimed to systematically assess the research landscape of traditional Chinese medicine (TCM) in the prevention and treatment of AD using bibliometric methods, with particular emphasis on its distinctive mechanisms and recent progress in clinical translation. **Methods**: Publications on TCM for AD prevention and treatment from 2015 to 2024 were retrieved from the Web of Science Core Collection. Key bibliometric indicators, including authorship, keywords, journals, countries, institutions, and references, were extracted and analyzed. **Results**: A total of 705 publications from 35 countries and 835 institutions were included. Among the top 10 contributing countries, 60% were located in Asia, while the rest were from North America, Europe, and Oceania. China led the field with 654 publications, accounting for 86.6% of the total output. The Journal of Ethnopharmacology emerged as the most influential journal based on citation count (848 citations). Keyword analysis highlighted molecular docking, neuroinflammation, and oxidative stress as key research hotspots. **Conclusion**: This bibliometric analysis offers valuable insights into global trends and research priorities regarding TCM in AD treatment. The findings provide a theoretical basis for promoting the clinical application of TCM and support the advancement of integrated efforts across industry, academia, and research sectors. The study also demonstrates enormous potential for cross-integration with perioperative medicine. Such integration is crucial for enhancing the role of TCM in the prevention and management of AD.

**Keywords**: Traditional Chinese medicine, Alzheimer's disease, bibliometrics, senile dementia

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## Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by insidious onset and progressive decline, primarily manifested as cognitive impairments in language, memory, executive function, judgment, and other domains [1-3]. According to the World Health Organization, an estimated 55 million individuals worldwide were living with AD in 2023. With the global population aging, this number is projected to reach 139 million by 2050, placing a substantial burden on patients, families, and healthcare systems [4]. Although the pathogenesis of AD remains unclear, prevailing hypotheses include the amyloid-beta (Aβ) cascade and tau protein hyperphosphorylation theories [5]. Additional contributing factors involve cholinergic neuronal degeneration, neuroinflammation caused by aberrant immune responses, and oxidative stress [6]. Current first-line treatments-such as cholinesterase inhibitors (rivastigmine, galantamine, donepezil) and N-methyl-D-aspartate receptor antagonists (memantine)-primarily act on the neurotransmitter system. While these drugs can temporarily improve symptoms, they do not prevent disease progression. Notably, under the "accelerated approval" pathway, the U.S. Food and Drug Administration approved two Aβ-targeted monoclonal antibodies: aducanumab in 2021 and lecanemab in 2023. Clinical trial data suggest that these agents can significantly slow cognitive decline in patients with early-stage AD [7-9]. However, their overall clinical benefit remains limited. In this context, the development of novel therapeutic strategies emphasizing multi-target and multi-mechanism approaches is gaining momentum.

With advances in science and technology and the deepening of research, the mechanisms underlying the therapeutic effects of TCM have been increasingly elucidated, contributing to its growing integration into modern healthcare systems [10-12]. TCM has demonstrated notable advantages in holistic regulation, reduced side effects, prevention-oriented care, multi-target therapeutic effects, chronic disease management, and modulation of inflammatory and immune responses. Furthermore, its emphasis on individualized treatment and systemic approaches has shown unique efficacy in managing chronic and complex diseases [13-16]. In recent years, an increasing number of researchers have investigated the application of TCM in the treatment of AD, resulting in significant progress and promising breakthroughs.

Bibliometrics is a research methodology that combines quantitative and qualitative analyses to evaluate the academic output and developmental status of specific research fields. By analyzing publication data, it provides detailed insights across multiple dimensions-such as authors, keywords, journals, countries, institutions, and references-thereby revealing research patterns, hotspots, and emerging trends within a discipline [17, 18]. Commonly used bibliometric tools include CiteSpace, VOSviewer, the "bibliometrix" package in R, and HistCite [19]. These tools facilitate the visualization of analytical results, allowing researchers to gain an intuitive understanding of the structure, evolution, and dynamics of a given research domain. In recent years, bibliometric studies related to AD have proliferated, addressing topics such as the comorbidity between AD and depression, as well as the application of biomarkers [20-24]. However, to date, no bibliometric analysis has specifically focused on the role of TCM in the prevention and treatment of AD. To fill this gap, the present study conducts a comprehensive bibliometric analysis of publications on TCM for AD from 2015 to 2024. The study aims to identify key contributors, map the current research landscape, and explore future trends and developmental prospects in this field.

## Methods

### *Search strategy*

The Web of Science Core Collection (https://www.webofscience.com/wos/woscc/basic-search) was selected as the data source for this study due to its comprehensive coverage of peer-reviewed journals. Search terms were determined based on the Medical Subject Headings database and included: "Chinese medicine","Chinese herbal medicine", "Chinese medicine prescription", "Chinese patent medicine", "Alzheimer's disease" and "senile dementia". The following search strategy was applied: (((TS=("Chinese medicine") OR TS=("Chinese herbal medicine")) OR TS=("Chinese medicine prescription") OR TS=("Chinese patent medicine"))) AND ((TS=("Alzheimer's disease") OR TS=("senile dementia"))). The search was conducted within the titles, abstracts, and keywords of eligible articles. Given the study's focus on the pharmacological effects of traditional Chinese mdicine—specifically herbal prescriptions, medicinal plants, and their active compounds—acupuncture-related terms were excluded from the search strategy. Only original research articles published in English between January 2015 and December 2024 were included. Non-English publications, conference

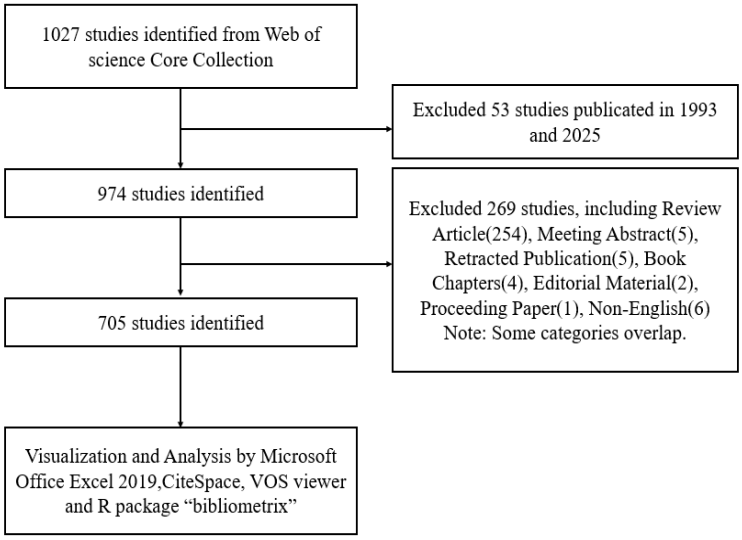


**Figure 1. Flowchart of publication screening.**

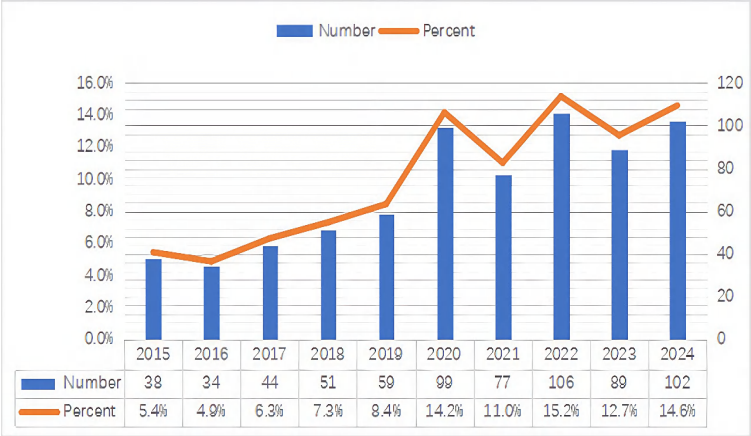


**Figure 2. Annual publication output.**

abstracts, book chapters, and retracted papers were excluded from the analysis. The detailed screening process is illustrated in **Figure 1**.

### *Data Analysis*

A range of specialized tools was employed for bibliometric analysis and data processing. VOSviewer (version 1.6.18), a widely used and efficient bibliometric analysis software, was utilized to extract key information from a large volume of publications. It is commonly applied to construct collaboration networks, co-citation networks, and keyword co-occurrence networks. In this study, VOSviewer was used to analyze countries and institutions, journals and co-cited journals, authors and co-cited authors, as well as keyword co-occurrence patterns. In the visual maps generated by VOSviewer, each node represents an item (e.g., country, institution, author, journal, or keyword), with node size corresponding to its frequency of occurrence. Links between nodes indicate associations such as collaboration or co-citation. The thickness of the lines reflects the strength of these relationships, and different colors distinguish between clusters or thematic groups [25].

CiteSpace (version 6.1.R1), developed by Professor Chaomei Chen, was employed primarily for citation burst analysis to identify influential references within the dataset [26].

Additionally, the R package "bibliometrix" (version 3.2.1; website: https://www.bibliometrix.org/) was used for thematic evolution analysis and to map global research distributions. Microsoft Excel 2019 was applied for quantitative analysis to ensure the accuracy and completeness of the dataset [27].

## Results

### *Quantitative analysis of publications*

A total of 705 studies related to the prevention and treatment of AD using TCM were identified over the past decade. Based on annual publication volume, the time frame was divided into two phases: Phase I (January 2015 to December 2019) and Phase II (January 2020 to December 2024). As illustrated in **Figure 2**, during Phase I, the average annual number of publications was approximately 45.2, with a relatively slow growth rate, indicating the early developmental stage of this research field. Notably, the year 2019 to 2020 marked the most significant annual increase in publication volume, rising from 59 to 99 articles—a year-on-year growth rate of 168%. In Phase II, the average annual publication output increased to 94.6, which is 2.1 times higher than that of Phase I. This trend reflects the growing academic attention and rapid advancement of research on TCM in the context of AD prevention and treatment.

### *Country and institutional analysis*

A total of 705 publications were retrieved, originating from 35 countries and 835 institutions. The top ten contributing countries were predominantly located in Asia (n=6) (**Table 1**),

**Table 1. Top 10 countries and institutions**

| Rank | Country | Counts | Institution | Counts |
|---|---|---|---|---|
| 1 | China (Asia) | 628 (83.2%) | Beijing University of Chinese Medicine (China) | 44 (17.5%) |
| 2 | UAS (North America) | 29 (3.8%) | China Academy of Chinese Medical Sciences (China) | 33 (13.1%) |
| 3 | Taiwan (Asia) | 26 (3.4%) | Guangzhou University of Chinese Medicine (China) | 32 (12.7%) |
| 4 | Japan (Asia) | 18 (2.4%) | Chinese Academy of Sciences (China) | 31 (12.3%) |
| 5 | South Korea (Asia) | 18 (2.4%) | Shenyang Pharmaceutical University (China) | 24 (9.5%) |
| 6 | Australia (Oceania) | 10 (1.3%) | Heilongjiang University of Chinese Medicine (China) | 19 (7.5%) |
| 7 | India (Asia) | 9 (1.2%) | Nanjing University of Chinese Medicine (China) | 18 (7.1%) |
| 8 | England (Europe) | 8 (1.1%) | Chengdu University of Traditional Chinese Medicine (China) | 17 (6.7%) |
| 9 | Pakistan (Asia) | 5 (0.7%) | University of Jinan (China) | 17 (6.7%) |
| 10 | Germany (Europe) | 4 (0.5%) | Tianjin University of Traditional Chinese Medicine (China) | 17 (6.7%) |

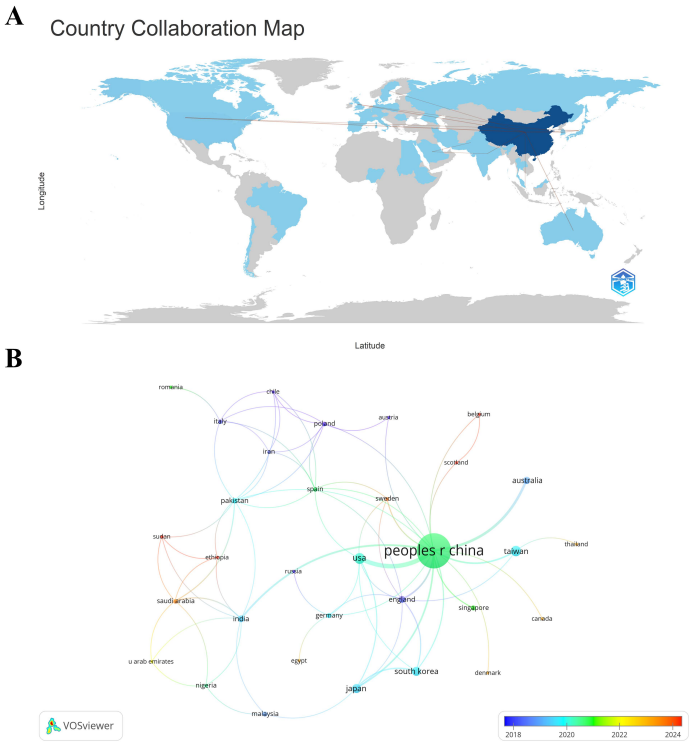


**Figure 3. Country and Institutional Analysis.** (A) Country Distribution; (B) Contry collaboration network.

with the remainder distributed across North America, Europe, and Oceania. Among them, China accounted for the overwhelming majority of publications (n=654, 86.6%), far exceeding all other countries. Based on the inclusion criterion of at least one publication, all 35 countries were included in a visualization map, and a country-level collaboration network was constructed according to publication volume and inter-country relationships (**Figure 3**). The results demonstrate that China maintains extensive collaborative ties, particularly with the United States, and also shows varying degrees of interaction with Australia, the United Kingdom, India, and Japan.

Among the contributing institutions, 225 had published at least two articles, the vast majority of which were located in China. The leading institutions by publication volume were: Beijing University of Chinese Medicine (n=44, 2.5%), China Academy of Chinese Medical Sciences (n=33, 1.9%), Guangzhou University of Chinese Medicine (n=32, 1.8%), Chinese Academy of Sciences (n=31, 1.8%), and Shenyang Pharmaceutical University (n=24, 1.4%). Institutions with at least eight publications (n=42) were selected for visualization and institutional collaboration network mapping (**Figure 4**).

The Peking University Health Science Center demonstrated strong collaborative relationships with the Guangzhou University of Chinese Medicine, the Chinese Academy of Sciences, and

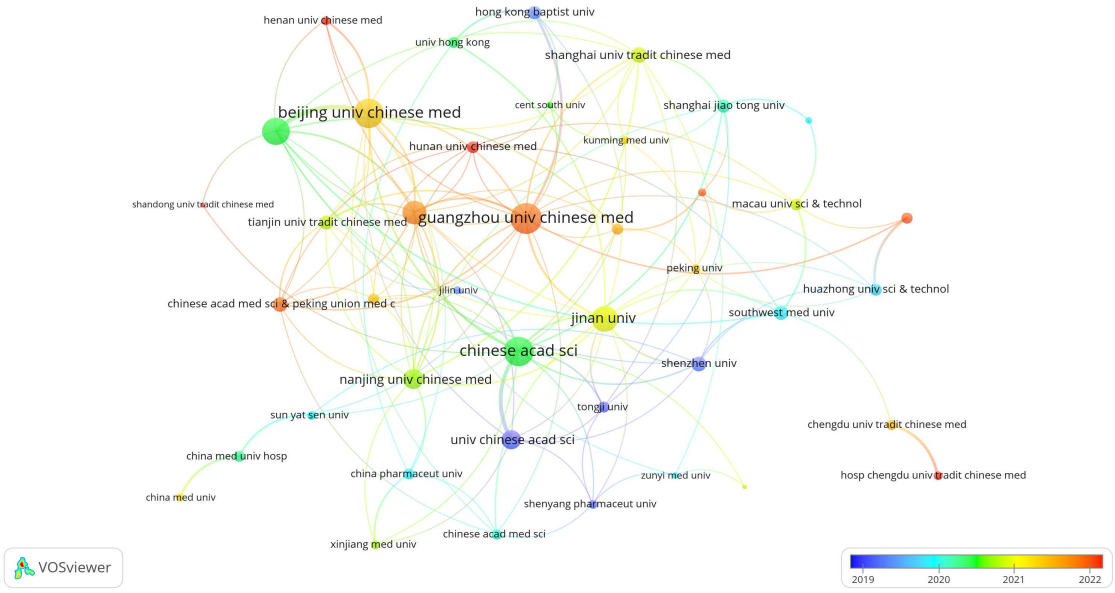


**Figure 4. Institutional collaboration network.**

**Table 2. Top 15 journals and co-cited journals**

| Rank | Journal | Count | IF | Q | Co-cited Journal | Co-citation | IF | Q |
|---|---|---|---|---|---|---|---|---|
| 1 | Journal of Ethnopharmacology | 67 (9.5%) | 4.8 | Q1 | Journal of Ethnopharmacology | 848 | 4.8 | Q1 |
| 2 | Frontiers in Pharmacology | 52 (7.4%) | 4.4 | Q1 | Journal of Alzheimer's Disease | 657 | 3.4 | Q2 |
| 3 | Evidence-based Complementary and Alternative Medicine | 32 (4.5%) | 2.7 | Q3 | PLOS ONE | 484 | 2.9 | Q1 |
| 4 | Phytomedicine | 18 (2.6%) | 6.7 | Q1 | Nucleic Acids Research | 428 | 16.6 | Q1 |
| 5 | Biomedicine & Pharmacotherapy | 14 (2.0%) | 6.9 | Q1 | Evidence-Based Complementary And Alternative Medicine | 410 | 2.7 | Q3 |
| 6 | Chinese Journal of integrative medicine | 14 (2.0%) | 2.2 | Q2 | Alzheimer's dement | 409 | 13 | Q1 |
| 7 | Frontiers in aging neuroscience | 14 (2.0%) | 4.1 | Q2 | Frontiers In Pharmacology | 406 | 4.4 | Q1 |
| 8 | Molecules | 13 (1.8%) | 4.2 | Q2 | International Journal of Molecular Sciences | 406 | 4.9 | Q1 |
| 9 | American journal of chinese medicine | 11 (1.6%) | 4.8 | Q1 | Journal of Neuroscience | 399 | 4.4 | Q1 |
| 10 | International journal of molecular sciences | 10 (1.4%) | 4.9 | Q1 | Scientific Reports | 365 | 3.8 | Q1 |
| 11 | Journal of alzheimers disease | 10 (1.4%) | 3.4 | Q2 | Molecules | 349 | 4.2 | Q2 |
| 12 | Neural regeneration research | 10 (1.4%) | 5.9 | Q1 | Proceedings of The National Academy of Sciences of The United States of America | 341 | 9.4 | Q1 |
| 13 | Food & Function | 9 (1.3%) | 5.1 | Q1 | Nature | 334 | 50.5 | Q1 |
| 14 | Bmc complementary medicine and therapies | 8 (1.1%) | 3.3 | Q1 | Journal of Biological Chemistry | 333 | 4.2 | Q2 |
| 15 | Current alzheimer research | 8 (1.1%) | 1.8 | Q4 | Neurobiology of Aging | 320 | 3.7 | Q2 |

Note: Q, quartile; IF, impact factor.

the China Academy of Chinese Medical Sciences. Notably, Heilongjiang University of Chinese Medicine, despit e its relatively high publication count, exhibited limited collaborative engagement with other institutions.

### *Journals and co-cited journals*

Research on the prevention and treatment of AD using TCM has been published across 235

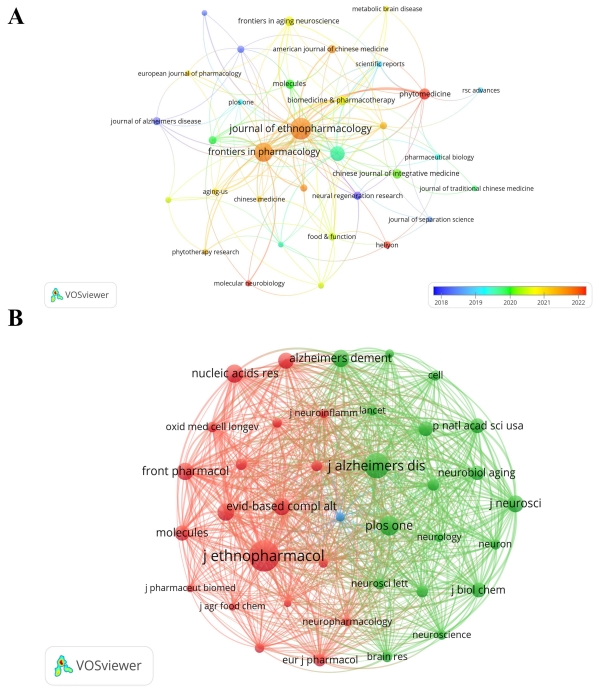


**Figure 5. Journals and Co-Cited Journals.** (A) Journal analytics visualization; (B) Co-cited journal analytics visualization.

academic journals. The Journal of Ethnopharmacology ranked first with 67 publications (9.5%), followed by Frontiers in Pharmacology (n=52, 7.4%), Evidence-Based Complementary and Alternative Medicine (n=32, 4.5%), and Phytomedicine (n=18, 2.6%). Among the top 15 journals, Biomedicine & Pharmacotherapy had the highest impact factor (IF=6.9), followed by Phytomedicine (IF=6.7) and Neural Regeneration Research (IF=5.9).

A total of 33 journals with at least five publications were selected to construct a journal citation network. As shown in **Figure 5A**, the Journal of Ethnopharmacology exhibited strong citation linkages with Phytomedicine, Frontiers in Pharmacology, and Evidence-Based Complementary and Alternative Medicine.

As presented in **Table 2**, three of the top 15 co-cited journals were cited more than 450 times. The most frequently co-cited journal was the Journal of Ethnopharmacology (co-citations =848), followed by the Journal of Alzheimer's Disease (n=657), PLOS ONE (n=484), and Nucleic Acids Research (n=428). The highest impact factor among these journals was observed for Nature (IF=50.5), followed by Nucleic Acids Research (IF=16.6). Journals with at least 150 co-citations were included to construct the co-citation network. As shown in **Figure 5B**, the Journal of Ethnopharmacology demonstrated strong co-citation relationships with the Journal of Alzheimer's Disease, PLOS ONE, and Nucleic Acids Research. The co-citation pattern between the Journal of Ethnopharmacology and the Journal of Alzheimer's Disease suggests that research in traditional medicine is increasingly intersecting with mainstream AD research, reflecting a growing methodological integration. The broad scope of PLOS ONE highlights the multidisciplinary nature of ethnopharmacological studies, while the connection with Nucleic Acids Research indicates a shift toward molecular-level investigations. Collectively, these findings underscore the progressive integration of ethnopharmacology into the forefront of modern biomedical science.

### *Authors and co-cited authors*

A total of 4,371 authors has contributed to research on the prevention and treatment of Alzheimer's disease using TCM. Among the top 10 most prolific authors, each has published at least seven articles, with the top two publishing ten or more. An author collaboration network was constructed based on individuals with at least four publications (**Figure 6A**). Notably, Fang JS, Li H, Liu ZQ, Wang Q, and Shi J have published a substantial number of studies and maintain close collaborative relationships.

Among the 20,161 co-cited authors, four have been cited more than 60 times (**Table 3**). The most frequently co-cited author is Selkoe DJ (n=79), followed by Ru JL (n=65), Scheltens P (n=64), and Wang Y (n=60). Authors with

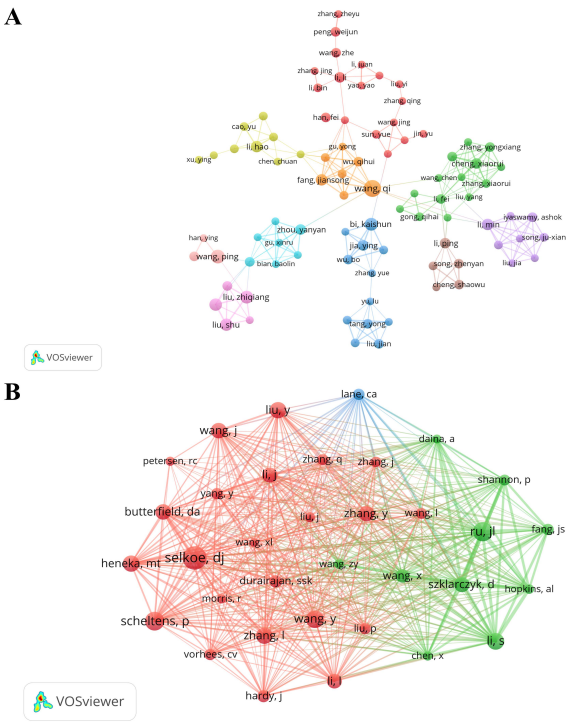


**Figure 6. Authors and Co-Cited Authors.** (A) Visualization of authors; (B) Visualization of co-cited authors.

**Table 3. Top 10 authors and co-cited authors**

| Rank | Authors | Count | Co-Cited Authors | Citations |
|---|---|---|---|---|
| 1 | Wang Q | 16 | Selkoe DJ | 79 |
| 2 | Wang P | 11 | Ru JL | 65 |
| 3 | Bi KS | 10 | Scheltens P | 64 |
| 4 | Liu ZQ | 10 | Wang Y | 60 |
| 5 | Liu S | 9 | Li S | 57 |
| 6 | Song FR | 9 | Heneka MT | 56 |
| 7 | Fang JS | 8 | Liu Y | 55 |
| 8 | Li H | 8 | Zhang L | 55 |
| 9 | Li M | 8 | Wang J | 53 |
| 10 | Cheng XR | 7 | Zhang Y | 53 |

at least 30 co-citations were included in the construction of the co-citation network (**Figure 6B**). As illustrated in **Figure 7**, there is active intellectual association among various co-cited authors, such as Selkoe DJ and Zhang Y, as well as Ru JL and Damian S.

### *Co-cited references*

From January 2015 to December 2025, a total of 28,679 co-cited references were identified in research on the prevention and treatment of Alzheimer's disease using TCM. Among the top 10 co-cited references (**Table 4**), each has been cited at least 25 times, with one reference cited more than 60 times (n=65). References with a minimum of 17 co-citations were selected to construct the co-citation network (**Figure 7**). As shown in **Figure 7**, "Ru JL, 2014, J Cheminformatics, v6" exhibits strong co-citation relationships with "Shannon P, 2003, Genome Res, v13, p2498" and "Li S, 2013, Chin J Nat Med, v11, p110" among others.

Several factors may explain the extensive citation of the article titled Clinical applications of the indirect immunofluorescence assay for detection of anticell membrane-associated DNA antibodies in juvenile systemic lupus erythematosus (Ru JL, 2014, J Cheminformatics, v6).

First, the Raji and HL60 cell lines compared in the study were later adopted in AD research to simulate neuroinflammatory environments. Second, the indirect immunofluorescence assay developed using Raji cells—owing to its high sensitivity and stability—may have been adapted by AD researchers for the detection of

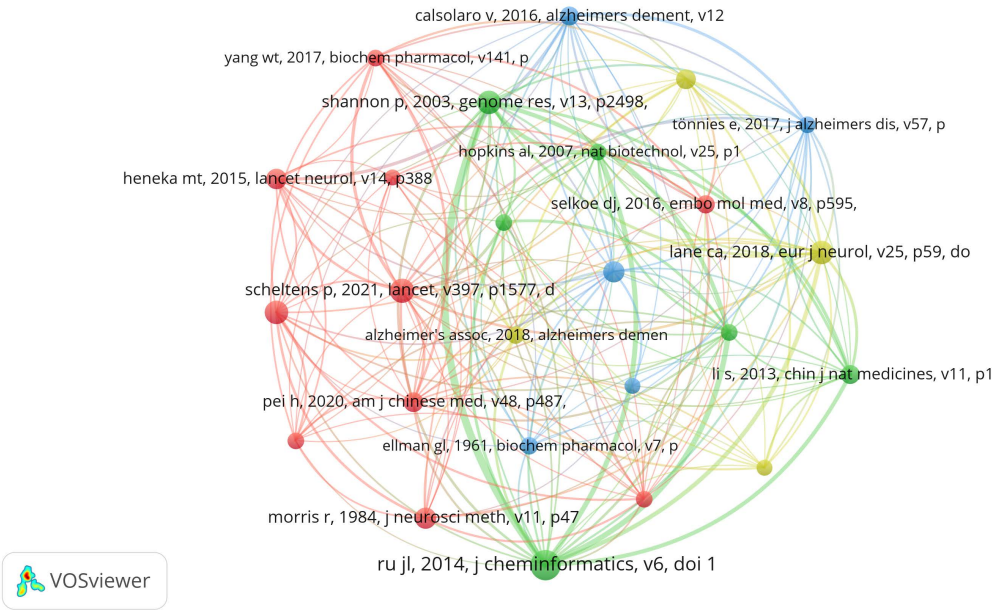


**Figure 7. Visualization of co-cited references.**

**Top 10 References with the Strongest Citation Bursts**

| References | Year | Strength | Begin | End | 2015 - 2024 |
|---|---|---|---|---|---|
| Scheltens P, 2016, LANCET, V388, P505, DOI 10.1016/S0140-6736(15)01124-1, DOI | 2016 | 5.56 | 2017 | 2020 | |
| Heneka MT, 2015, LANCET NEUROL, V14, P388, DOI 10.1016/S1474-4422(15)70016-5, DOI | 2015 | 4.67 | 2018 | 2020 | |
| Ru JL, 2014, J CHEMINFORMATICS, V6, P0, DOI 10.1186/1758-2946-6-13, DOI | 2014 | 3.95 | 2018 | 2019 | |
| Kumar A, 2015, PHARMACOL REP, V67, P195, DOI 10.1016/j.pharep.2014.09.004, DOI | 2015 | 3.81 | 2018 | 2020 | |
| Alzheimers Assoc, 2018, ALZHEIMERS DEMENT, V14, P367, DOI 10.1016/j.jalz.2018.02.001, DOI | 2018 | 3.73 | 2018 | 2020 | |
| Selkoe DJ, 2016, EMBO MOL MED, V8, P595, DOI 10.15252/emmm.201606210, DOI | 2016 | 5.83 | 2019 | 2020 | |
| Yang WT, 2017, BIOCHEM PHARMACOL, V141, P143, DOI 10.1016/j.bcp.2017.07.002, DOI | 2017 | 3.87 | 2019 | 2020 | |
| Jarrell JT, 2018, MOLECULES, V23, P0, DOI 10.3390/molecules23051143, DOI | 2018 | 4.7 | 2021 | 2022 | |
| Lopez JAS, 2019, HAND CLINIC, V167, P231, DOI 10.1016/B978-0-12-804766-8.00013-3, DOI | 2019 | 4.34 | 2022 | 2024 | |
| Pei H, 2020, AM J CHINESE MED, V48, P487, DOI 10.1142/S0192415X20500251, DOI | 2020 | 4.14 | 2022 | 2024 | |

**Figure 8. Top 10 references with strong citation bursts.**

**Table 4. Top 10 co-cited references**

| Rank | Co-cited reference | Citations |
|---|---|---|
| 1 | Ru JL, 2014, J Cheminformatics, v6 (28) | 65 |
| 2 | Scheltens P, 2021, Lancet, v397, p1577 | 39 |
| 3 | Lane CA, 2018, Eur J Neurol, v25, p59 | 37 |
| 4 | Shannon P, 2003, Genome Res, v13, p2498 | 37 |
| 5 | Vorhees CV, 2006, Nat Protoc, v1, p848 | 37 |
| 6 | Morris R, 1984, J Neurosci Meth, v11, p47 | 31 |
| 7 | Hardy J, 2002, Science, v297, p353 | 30 |
| 8 | Heneka MT, 2015, Lancet Neurol, v14, p388 | 29 |
| 9 | Pei H, 2020, Am J Chinese Med, v48, p487 | 25 |
| 10 | Scheltens P, 2016, Lancet, v388, p505 | 25 |

central nervous system-specific autoantibodies. Lastly, the impaired clearance mechanism of cell membrane-associated DNA described in the study, particularly the dysfunction of the 30 kDa membrane receptor, may provide a conceptual reference for analogous autoimmune phenomena in observed AD, such as impaired β-amyloid protein clearance.

### *References with citation bursts*

References with citation bursts are those that have been frequently within a specific timeframe, indicating emerging or transient scholarly interest. In this study, ten references demonstrated strong citation bursts, as identified using CiteSpace software (**Figure 8**). In the visualization, red bars represent periods of high ci-

**Table 5. Main research content of the top 10 references with strong citation bursts**

| Rank | Strength | Main research content |
|---|---|---|
| 1 | 5.56 | Overview of major developments in Alzheimer's disease research [28]. |
| 2 | 4.67 | Characterization of the neuroinflammatory landscape in Alzheimer's disease [29]. |
| 3 | 3.95 | Use of the TCMSP database to construct drug-target and drug-disease networks, facilitating the development of novel herb-based therapeutics [30]. |
| 4 | 3.81 | Exploration of various pathophysiological mechanisms underlying Alzheimer's disease [31]. |
| 5 | 3.73 | Assessment of the public health impact of Alzheimer's disease [32]. |
| 6 | 5.83 | Summary of three decades of key findings supporting the amyloid hypothesis, along with discussion of alternative perspectives and challenges [33]. |
| 7 | 3.87 | Evaluation of the efficacy and safety of Chinese herbal medicine for Alzheimer's disease, with exploration of its role in promoting neurogenesis [34]. |
| 8 | 4.7 | Investigation into the potential of traditional Chinese medicine in treating Alzheimer's disease through network pharmacology approaches [35]. |
| 9 | 4.43 | Review of recent advances in Alzheimer's disease research [36]. |
| 10 | 4.14 | Demonstration of the effectiveness of traditional Chinese herbs in improving cognitive dysfunction [37]. |

Note: TCMSP, Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform.

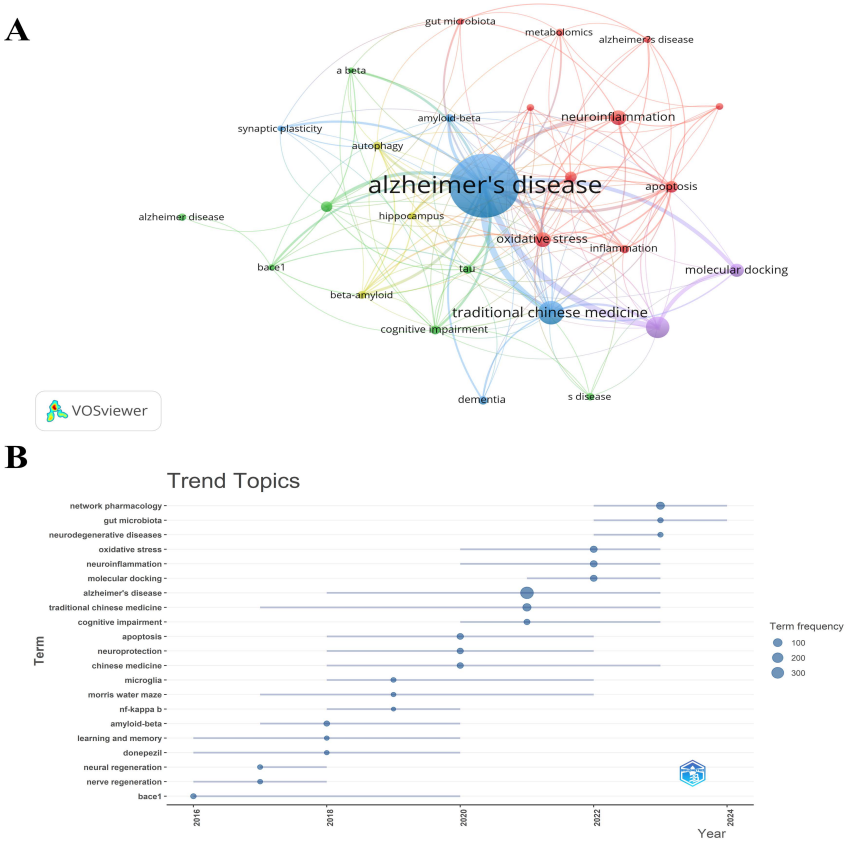


**Figure 9. Hotspots and Frontiers.** (A) Visualization of keyword cluster analysis; (B) Trend topic analysis.

tation burst intensity. The earliest citation burst occurred in 2017, and the most recent in 2024. The reference with the strongest citation burst (strength=5.83) is The amyloid hypothesis of Alzheimer's disease at 25 years" by Selkoe et al., with a burst period from 2019 to 2020. The second strongest burst (strength= 5.56) is associated with "Alzheimer's disease", published in The Lancet by Philip S et al., spanning the period from 2017 to 2020. Overall, the burst strength of these ten references ranged from 3.73 to 5.83, with durations varying between 2 and 4 years. **Table 5** provides a summary of the key research content of these references, listed in the same order as shown in **Figure 8** [28-37].

### *Hotspots and frontiers*

Keyword co-occurrence analysis enables the rapid identification of research hotspots within

**Table 6. Top 20 keywords**

| Rank | Keyword | Count | Rank | Keyword | Count |
|---|---|---|---|---|---|
| 1 | Alzheimer's disease | 361 | 11 | Dementia | 17 |
| 2 | Traditional Chinese medicine | 78 | 12 | Tau | 17 |
| 3 | Network pharmacology | 68 | 13 | Inflammation | 16 |
| 4 | Oxidative stress | 39 | 14 | Amyloid-beta | 15 |
| 5 | Neuroinflammation | 38 | 15 | Autophagy | 15 |
| 6 | Molecular docking | 32 | 16 | Beta-amyloid | 15 |
| 7 | Apoptosis | 27 | 17 | Alzheimer disease | 14 |
| 8 | Chinese medicine | 23 | 18 | Metabolomics | 14 |
| 9 | Neuroprotection | 23 | 19 | Senile disease | 13 |
| 10 | Cognitive impairment | 18 | 20 | Acetylcholinesterase | 12 |

a specific field. **Table 6** presents the top 20 high-frequency keywords in studies on the prevention and treatment of AD using TCM. Among these, "Alzheimer's disease", "traditional Chinese medicine" and "network pharmacology" appear more than 60 times, highlighting the primary research directions in this area. Keywords with at least 10 occurrences were selected for cluster analysis using VOSviewer (**Figure 9A**). In the visualization, thicker lines indicate stronger connections between keywords.

As shown in **Figure 9A**, four major clusters were identified. The blue cluster includes keywords such as "Alzheimer's disease", "traditional Chinese medicine", "dementia", "synaptic plasticity" and "amyloid-beta". The red cluster features keywords such as "neuroinflammation", "oxidative stress", "apoptosis", "metabolomics" and "acetylcholinesterase". The purple cluster includes "network pharmacology" and "molecular docking". The green cluster contains terms such as "Chinese medicine", "cognitive impairment", "amyotrophic lateral sclerosis" and "tau protein."

Trend topic analysis (**Figure 9B**) indicates that from 2015 to 2020, research primarily focused on neurocognitive aspects, with keywords such as "neuroregeneration", "learning and memory", "neuroprotection", "amyloid-beta", "nuclear factor kappa B", "Morris water maze" and "microglia." After 2020, scholars increasingly explored TCM-based interventions for AD using techniques such as network pharmacology and molecular docking. Representative keywords during this period include "traditional Chinese medicine", "Alzheimer's disease", "molecular docking", "neuroinflammation", "oxidative stress", "neurodegenerative diseases", "gut microbiota" and "network pharmacology."

Recent advancements in systems biology and computational tools have facilitated the emergence of innovative approaches—such as network pharmacology and molecular docking—for deciphering the multi-target mechanisms of TCM against AD. Moving beyond the single-target paradigm, these strategies construct comprehensive "component-target-pathway" networks that systematically elucidate the synergistic effects of TCM compounds [38,39]. This integrated research framework—combining bioinformatics, computational chemistry, and experimental validation—has not only revealed the molecular mechanisms underlying classical TCM formulations (e.g., Huanglian Jiedu Decoction and Danggui Shaoyao San) in modulating neuroinflammation, oxidative stress, and synaptic plasticity, but has also provided a theoretical foundation and direction for the development of novel TCM-based anti-AD drugs. Furthermore, the frequent appearance of neuroinflammation and oxidative stress as keywords suggests that these remain central research hotspots in the treatment of AD.

## Discussion

Between 2015 and 2019, research on the prevention and treatment of AD using TCM remained in the exploratory stage, with an average annual publication output of 45.2 articles. However, from 2020 to 2024, the field witnessed a substantial surge in activity, with the average annual number of publications rising to 94.6-representing an impressive 109% increase. This rapid growth indicates that TCM-based AD research has entered a phase of accelerated development and is gradually becoming a focus of international scientific attention. The significant expansion of this research area has been closely aligned with China's strategic initiatives for TCM development. In particular, the 14th Five-Year Plan for the Development of Traditional Chinese Medicine, issued by the General Office of the State Council in 2022, explicitly called for a substantial enhancement of TCM healthcare service capacity and the establishment of a high-quality development pol-

icy framework by 2025. Driven by strong policy support, the unique advantages of TCM in the prevention and treatment of AD have been fully harnessed, resulting in notable advancements in both research scale and quality.

These advancements have laid a solid foundation for the innovative development of TCM in the field of neurodegenerative diseases. Currently, TCM-based research on AD is rapidly transitioning from basic investigation to clinical translation, highlighting its promising prospects for future development.

The systematic advancement of modernization strategies for TCM has played a pivotal role in shaping an international framework for scientific cooperation. In building a high-level TCM system for the preservation and innovation of TCM, China has implemented a series of comprehensive measures. Notably, a specialized national program for the inheritance of ancient TCM literature has been carried out, resulting in the development of a traditional knowledge database encompassing 32,000 volumes of classical texts. In addition, the strategic establishment of 30 national TCM inheritance and innovation centers, along with 15 interdisciplinary national key laboratories, has contributed to the construction of a complete research and innovation chain.

Notably, this systematic infrastructure development has laid a strong foundation for international collaboration. Statistical evidence indicates that the majority of co-authored publications in TCM-based neurodegenerative disease research are jointly produced by institutions in China and the United States. Moreover, collaborative publications involving Australia, the United Kingdom, India, and Japan have shown a steady upward trend.

In terms of institutional cooperation networks, a stable collaborative cluster-resembling an academic community-has emerged, centered around Beijing University of Chinese Medicine and the China Academy of Chinese Medical Sciences, with more than 40% of their publications involving international collaboration. However, comparative analysis reveals that although Heilongjiang University of Chinese Medicine ranks among the top institutions with an annual output of 27 publications, its international collaboration rate remains at only 12%. This “high-output-low-collaboration” model may hinder the sustained improvement of research quality.

This phenomenon underscores the need to establish more open and inclusive academic cooperation mechanisms to promote positive interactions between cultural dissemination and technological innovation. Such efforts are essential for advancing the strategic objective outlined in the 14th Five-Year Plan for the Development of Traditional Chinese Medicine-namely, to significantly enhance the international influence of TCM.

With regard to research on TCM for AD, the Journal of Ethnopharmacology [IF=4.8, quartile (Q) 1] has published the highest number of articles, indicating its prominence as a leading journal in this research domain. Among the publishing journals, Biomedicine & Pharmacotherapy (IF=6.9, Q1) holds the highest impact factor, followed by Phytomedicine (IF =6.7, Q1). In terms of co-cited journals, the majority are high-impact Q1 journals, reflecting the academic influence and credibility of the research in this field. An analysis of current clinical research on TCM for the prevention and treatment of AD reveals a clear imbalance between basic and clinical studies. While significant advancements have been achieved in basic research-with 83.2% of publications focused on mechanistic exploration-clinical translation remains relatively limited.

At present, clinical studies account for fewer than 5% of total publications, and rigorously designed trials are even scarcer. According to data from the Chinese Clinical Trial Registry, several representative clinical trials investigating TCM interventions for AD have been registered. These include: (1) A study of Polygonum cuspidatum Yizhi Granules for mild cognitive impairment due to AD (ChiCTR2400081422); (2) A randomized, double-blind, placebo-controlled trial of Zhinao Capsule for mild-to-moderate AD (ChiCTR2200066501); (3) A clinical observation of Nourishing Blood Oral Liquid on cognitive function in AD patients with Qi-blood deficiency type (ChiCTR2400087425) (https://www.chictr.org.cn/question.html). Notably, the Polygonum cuspidatum Yizhi Granules study represents an innovative integration of modern neuroscience theories and technologies with TCM therapeutic principles. By employing high-quality randomized controlled trial methodology, this study systematically evaluates the efficacy of the intervention for mild cognitive impairment due to AD, potentially offering novel therapeutic strategies. It exemplifies the modernization of TCM research for mild cognitive impairment due to AD, wherein the combined advantages of integrative medicine are being harnessed to establish a clinical diagnosis and treatment framework with distinct Chinese characteristics. Such efforts are expected to

yield more effective and internationally recognized treatment protocols.

From the perspective of authorship, Fang JS, Bi KS, Song FR, and Li H contributed a substantial number of publications, with an average of 9.75 articles per author. Further analysis and discussion were conducted based on the works of these most prolific scholars.

Fang et al. have employed systems pharmacology and network pharmacology approaches to investigate the mechanisms of action of various TCM formulations and their active components in the treatment of AD [40, 41]. In 2021, they screened cytochrome P450 3A4 inhibitors from the Bushen Yizhi Formula to enhance the bioavailability of osthole [42]. In the same year, they explored the ability of medicarpin to alleviate scopolamine-induced memory impairment using systems pharmacology methods [43]. Subsequently, by integrating proteomics and metabolomics, they elucidated the molecular mechanisms underlying the effects of Danggui Shaoyao San in amyloid precursor protein/presenilin-1 mice, and identified TCM-derived active compounds that target endoplasmic reticulum stress through network analysis [44]. In 2023, they conducted a comprehensive systems pharmacological evaluation using multi-network models and preliminarily identified the effective material basis and potential molecular mechanisms of kidney-tonifying TCMs for the prevention and treatment of AD via endophenotype network analysis [45]. Additionally, Fang et al. assessed the predictive capacity of the Target Knowledge Graph knowledge graph to identify potential protein targets for AD and to uncover diseases potentially associated with the metallodeubiquitinase COP9 signalosome subunit 5, validating the predictions through literature-based evidence [46]. Although these research findings have not yet to been translated into clinical applications, the exploratory nature of this body of work is highly representative. The research trajectory from 2017 to 2023 reflects the evolving landscape of TCM-based research—progressing from compound identification and mechanistic elucidation to the investigation of systemic regulatory mechanisms.

In terms of co-cited authors, Dennis JS (citation count=79) is the most frequently cited, followed by Ru JL (citation count=65) and Scheltens P (citation count=64). In 2016, Dennis JS reviewed the amyloid hypothesis, emphasizing the central role of Aβ in the early pathogenesis of AD and identifying abnormal Aβ accumulation as a key hallmark of the disease [33]. An earlier study elucidated the tripeptide cleavage mechanism of γ-secretase in Aβ production, thereby explaining Aβ formation at the molecular level and offering a theoretical foundation for the development of γ-secretase-targeted therapies [47]. Subsequent research demonstrated that large soluble Aβ oligomers possess lower neuroactivity, whereas smaller oligomers exhibit higher neurotoxicity, indicating that both the morphology and size of Aβ oligomers significantly influence their toxic potential. This finding provided a novel perspective on the pathological mechanisms of Aβ [48]. In 2019, clinical trial outcomes of aducanumab—a monoclonal antibody targeting Aβ-were reported, underscoring the need to refine AD treatment strategies and integrate anti-amyloid therapies with other modalities [49]. Collectively, these studies advanced the understanding of Aβ's role in AD and laid a solid scientific foundation for future diagnostic and therapeutic strategies. The amyloid cascade hypothesis was systematically re-evaluated, with emphasis on abnormal Aβ deposition as an early pathological indicator of AD. Aβ oligomers were proposed as the primary neurotoxic species, providing a rationale for Aβ-targeted drug development. Clinically, Aβ-positron emission tomography imaging and cerebrospinal fluid Aβ42 detection have been established as early diagnostic criteria for AD, thereby facilitating the development of anti-Aβ monoclonal antibodies such as aducanumab and lecanemab.

Co-cited references refer to publications that are simultaneously cited by multiple independent articles and are therefore considered to represent the theoretical or knowledge foundation of a particular research field. Below is a brief summary of the five most frequently co-cited references.

Ru et al. introduced the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform, which encompasses all herbal medicines listed in the Chinese Pharmacopoeia and nearly 30,000 extracted compounds [30]. The platform provides 12 key absorption, distribution, metabolism, and excretion-related properties for drug screening and evaluation, along with information on drug targets and associated diseases for each active compound. In addition, it can automatically construct compound-target and target-disease networks, enabling users to visualize and analyze the mechanisms of drug action. Overall, the platform is designed to promote the development of herbal medicine and facilitate the integration of modern and traditional medicine to advance drug discovery and development.

Scheltens et al. summarized major advances in the field of AD over the past five years, covering clinical signs and symptoms, incidence and mortality rates, causative and risk genes, pathophysiological features, and biomarkers [29]. In 2017, Lane et al. reviewed the clinical manifestations of AD and current treatment strategies, providing an overview of the prevailing understanding of AD epidemiology, genetics, pathology, and pathogenesis [50]. Their work highlighted the extended preclinical phase of AD and its implications for novel therapeutic strategies aimed at shifting the focus from treatment to prevention. Shannon et al. explored molecular, clinical, and neuropathological networks related to viral activity, aligning with the hypothesis that viral processes may underlie general characteristics of AD [51]. By constructing multi-scale virome networks associated with late-onset AD and integrating genomic, transcriptomic, proteomic, and histopathological data from four brain regions of postmortem human tissues, they identified regulatory relationships between viral abundance and amyloid precursor protein metabolic regulators. Collectively, these three publications provided systematic reviews of research progress in AD. However, due to their relatively early publication dates, the current study does not include in-depth interpretation or critical analysis of their findings.

Vorhees et al. systematically introduced the Morris Water Maze, a widely used spatial learning test for rodents [52]. In this paradigm, animals use distal environmental cues to navigate from various starting points within an open swimming arena to locate a submerged escape platform. Key experimental metrics such as escape latency and platform crossing frequency are employed to assess spatial cognitive function. Recognized as a "behavioral cornerstone" in AD research, Morris Water Maze has yielded robust phenotypic data for elucidating pathological mechanisms and facilitating drug screening. Moreover, through continuous technological innovations, the test has driven paradigm shifts in AD research-advancing from phenomenological observation to mechanistic exploration, and from single-target interventions to systemic therapeutic strategies. This methodological approach is considered to hold significant promise for broader applications in the study of neurological disorders.

In addition to references with citation bursts, keyword analysis serves as an effective tool for identifying the distribution and evolution of research hotspots related to the prevention and treatment of AD using TCM. Beyond general terms such as "Alzheimer's disease", "traditional Chinese medicine" and "Chinese herbs". **Table 6** highlights several frequently occurring keywords, including "network pharmacology","oxidative stress", "neuroinflammation", "molecular docking" and "apoptosis". Based on keyword clustering and trending topic analysis (**Figure 9**), the current research on TCM-based prevention and treatment of AD is primarily concentrated in the following thematic aspects.

Firstly, the potential etiology and pathophysiological characteristics of AD have been a major focus. The etiology of AD is multifactorial, involving genetic, environmental, metabolic, and immune components. Based on the cited literature, research has primarily concentrated on key pathogenic mechanisms such as Aβ deposition, abnormal phosphorylation of tau protein, neuroinflammation, oxidative stress, and synaptic dysfunction. These pathological processes result in substantial neuronal loss in the hippocampus and cerebral cortex, ultimately leading to brain atrophy and progressive cognitive decline, which may advance to severe dementia. Secondly, the docking of target molecules and the discovery of novel pathways are central themes [53, 54]. Network pharmacology elucidates the multi-target mechanisms of therapeutic agents by constructing drug-target-disease interaction networks, while molecular docking simulates the binding patterns between drug molecules and target proteins to predict their interactions and affinities. Together, these approaches offer substantial advantages in drug discovery, mechanistic research, and the advancement of personalized medicine.

### Strengths and limitations

This study presents several notable strengths. First, it is the first to conduct a systematic bibliometric analysis of research on AD involving TCM, offering scholars comprehensive guidance for identifying and focusing on relevant literature. Second, three bibliometric tools were employed concurrently—two of which, VOSviewer and CiteSpace, are widely recognized and commonly used in bibliometric research—enhancing the objectivity and reliability of the data analysis process. Third, compared to traditional narrative reviews, the bibliometric approach provides deeper insights into research hotspots and emerging frontiers.

Nonetheless, several limitations should be acknowledged. First, the data were exclusively retrieved from the Web of Science Core Collec-

tion, while other databases were not included, which may have led to the omission of relevant studies. Second, only English-language publications were considered, potentially resulting in the underrepresentation of non-English literature. Finally, publications from the year 2025 were not included in the current analysis.

## Conclusions

TCM holds substantial research value and promising application prospects in the prevention and treatment of AD. The rapid growth in publication volume reflects increasing global interest in the therapeutic potential of TCM, with China playing a leading role. However, greater international collaboration and communication among countries and institutions are still needed.

On one hand, investigations into the pathogenesis and contributing factors of AD offer critical insights for early diagnosis and intervention, while also providing a theoretical foundation for the development of novel drugs and therapeutic strategies. On the other hand, compared to conventional chemical drugs, TCM demonstrates significant advantages, including holistic regulation, multi-target efficacy, and anti-inflammatory immune modulation. Furthermore, TCM emphasizes personalized treatment and a holistic perspective, showcasing unique effectiveness in managing AD.

It is important to highlight that, beyond basic research, future efforts should focus on the clinical translation of research findings, particularly regarding the application of TCM in the prevention and treatment of AD.

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## References

[1] Jannat K, Balakrishnan R, Han JH, et al. The Neuropharmacological Evaluation of Seaweed: A Potential Therapeutic Source. Cells 2023;12(22):2652.

[2] DeTure MA, Dickson DW. The neuropathological diagnosis of Alzheimer's disease. Mol Neurodegener 2019;14(1):32.

[3] Jack CR Jr, Bennett DA, Blennow K, et al. NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. Alzheimers Dement 2018;14(4):535-562.

[4] Alzheimer's Disease International. World Alzheimer Report 2023. Available at: https://www.alzint.org/resource/world-alzheimer-report-2023/#:~:text=The%20World%20Alzheimer%20Report%202023%2C%20written%20in%20journalistic,dementia%20risk%20factors%20are%20experienced%20around%20the%20world.

[5] Mu Y, Zhao HM, Liu HC, et al. Advances in drug development for Alzheimer's disease. J China Pharm Univ 2024;55(6):816-825.

[6] Alzheimer's Disease Branch of China Association of Geriatric Health and Brain Disease Drug Research Professional Committee of China Association of Traditional Chinese Medicine. Chinese Expert Consensus on Integrated Traditional Chinese and Western Medicine Diagnosis and Treatment for Alzheimer's Disease. Chin J Behav Med Brain Sci 2024;33(2):97-108.

[7] Zhang J, Zhang Y, Wang J, et al. Recent advances in Alzheimer's disease: Mechanisms, clinical trials and new drug development strategies. Signal Transduct Target Ther 2024;9(1):211.

[8] Singh A, Kukreti R, Saso L, et al. Oxidative Stress: A Key Modulator in Neurodegenerative Diseases. Molecules 2019;24(8):1583.

[9] Du MF, Xu B, Guo DY, et al. Research Progress on Traditional Chinese Medicine for Alzheimer's Disease Based on Aβ Protein. J Shaanxi Univ Chin Med 2022;45(2):146-150.

[10] Zhao J, Lan X, Liu Y, et al. Anti-aging role of Chinese herbel medicine: an overview of scientific evidence from 2008 to 2018. Ann Palliat Med 2020;9(3):1230-1248.

[11] Wu X, Hu X, Zhang Q, et al. Regulatory Role of Chinese Herbal Medicine in Regulated Neuronal Death. CNS Neurol Disord Drug Targets 2021;20(3):228-248.

[12] Wang ZY, Liu JG, Li H, et al. Pharmacological Effects of Active Components of Chinese Herbal Medicine in the Treatment of Alzheimer's Disease: A Review. Am J Chin Med 2016;44(8):1525-1541.

[13] Jarrell JT, Gao L, Cohen DS, et al. Network Medicine for Alzheimer's Disease and Traditional Chinese Medicine. Molecules 2018;23(5):1143.

[14] Ding MR, Qu YJ, Hu B, et al. Signal pathways in the treatment of Alzheimer's disease with traditional Chinese medicine. Biomed Pharmacother 2022;152:113208.

[15] Zhou XB, Zhang YX, Zhou CX, et al. Chinese Herbal Medicine Adjusting Brain Microenviron-

ment via Mediating Central Nervous System Lymphatic Drainage in Alzheimer's Disease. Chin J Integr Med 2022;28(2):176-184.
[16] Jiao L, Bi L, Lu Y, et al. Cancer chemoprevention and therapy using chinese herbal medicine. Biol Proced Online 2018;20:1.
[17] Ellegaard O, Wallin JA. The bibliometric analysis of scholarly production: How great is the impact? Scientometrics 2015;105(3):1809-1831.
[18] Martín-Martín A, Thelwall M, Orduna-Malea E, et al. Google Scholar, Microsoft Academic, Scopus, Dimensions, Web of Science, and OpenCitations' COCI: a multidisciplinary comparison of coverage via citations. Scientometrics 2021;126(1):871-906.
[19] Ninkov A, Frank JR, Maggio LA. Bibliometrics: Methods for studying academic publishing. Perspect Med Educ 2022;11(3):173-176.
[20] Synnestvedt MB, Chen C, Holmes JH. CiteSpace II: visualization and knowledge discovery in bibliographic databases. AMIA Annu Symp Proc 2005;2005:724-728.
[21] Yeung AW, Goto TK, Leung WK. The Changing Landscape of Neuroscience Research, 2006-2015: A Bibliometric Study. Front Neurosci 2017;11:120.
[22] Yang L, Zeng J, Li L, et al. Biomarkers and Alzheimer's disease: a bibliometric analysis. Front Aging Neurosci 2024;16:1456824.
[23] Sun W, Gong J, Li S, et al. Bibliometric analysis of neuroinflammation and Alzheimer's disease. Front Aging Neurosci 2024;16:1423139.
[24] Jun P, Chengye H, Hui W. Bibliometric analysis of rehabilitation in Alzheimer's disease (2000-2023): trends, hotspots and prospects. Front Aging Neurosci 2024;16:1457982.
[25] van Eck NJ, Waltman L. Software survey: VOSviewer, a computer program for bibliometric mapping. Scientometrics 2010;84(2):523-538.
[26] Arruda H, Silva ER, Lessa M, et al. VOSviewer and Bibliometrix. J Med Libr Assoc 2022;110(3):392-395.
[27] Pan XL, Yan EJ, Cui M, et al. Examining the usage, citation, and diffusion patterns of bibliometric mapping software: A comparative study of three tools. J Informetr 2018;12(2):481-493.
[28] Scheltens P, De Strooper B, Kivipelto M, et al. Alzheimer's disease. Lancet 2021;397(10284):1577-1590.
[29] Heneka MT, Carson MJ, El Khoury J, el al. Neuroinflammation in Alzheimer's disease. Lancet Neurol 2015 Apr;14(4):388-405.
[30] Ru J, Li P, Wang J, et al. TCMSP: a database of systems pharmacology for drug discovery from herbal medicines. J Cheminform 2014;6:13.
[31] Kumar A, Singh A, Ekavali. A review on Alzheimer's disease pathophysiology and its management: an update. Pharmacol Rep 2015 Apr;67(2):195-203.
[32] Alzheimer's assoc, 2018 Alzheimer's disease facts and figures. 2018, Alzheimers dement 14(3): 367-425.
[33] Selkoe DJ, Hardy J. The amyloid hypothesis of Alzheimer's disease at 25 years. EMBO Mol Med. 2016 Jun 1;8(6):595-608.
[34] Yang WT, Zheng XW, Chen S, el al. Chinese herbal medicine for Alzheimer's disease: Clinical evidence and possible mechanism of neurogenesis. Biochem Pharmacol. 2017 Oct 1;141:143-155.
[35] Jarrell JT, Gao L, Cohen DS, el al. Network Medicine for Alzheimer's Disease and Traditional Chinese Medicine. Molecules 2018 May 11;23(5):1143.
[36] Soria Lopez JA, González HM, Léger GC. Alzheimer's disease. Handb Clin Neurol 2019;167:231-255.
[37] Pei H, Ma L, Cao Y, el al. Traditional Chinese Medicine for Alzheimer's Disease and Other Cognitive Impairment: A Review. Am J Chin Med 2020;48(3):487-511.
[38] Wang X, Zhang Y, Niu H, et al. Ultra-fast liquid chromatography with tandem mass spectrometry determination of eight bioactive components of Kai-Xin-San in rat plasma and its application to a comparative pharmacokinetic study in normal and Alzheimer's disease rats. J Sep Sci 2017;40(10):2131-2140.
[39] Guo S, Wang J, Wang Y, et al. Study on the Multitarget Synergistic Effects of Kai-Xin-San against Alzheimer's Disease Based on Systems Biology. Oxid Med Cell Longev 2019;2019:1707218.
[40] Pang XC, Kang D, Fang JS, et al. Network pharmacology-based analysis of Chinese herbal Naodesheng formula for application to Alzheimer's disease. Chin J Nat Med 2018;16(1):53-62.
[41] Luo Y, Li D, Liao Y, et al. Systems Pharmacology Approach to Investigate the Mechanism of Kai-Xin-San in Alzheimer's Disease. Front Pharmacol 2020;11:381.
[42] Liu JM, Chen JM, Lin MJ, et al. Screening and verification of CYP3A4 inhibitors from Bushen-Yizhi formula to enhance the bioavailability of osthole in rat plasma. J Ethnopharmacol 2022;282:114643.
[43] Li D, Cai C, Liao Y, et al. Systems pharmacology approach uncovers the therapeutic mechanism of medicarpin against scopolamine-induced memory loss. Phytomedicine 2021;91:153662.
[44] Wu Q, Wang W, Huang Z, et al. Unveiling the molecular mechanisms of Danggui-Shaoyao-San against Alzheimer's disease in APP/PS1 mice via integrating proteomic and me-

tabolomic approaches. Alzheimers Res Ther 2024;16(1):251.

[45] Dai Z, Hu T, Wei J, et al. Network-based identification and mechanism exploration of active ingredients against Alzheimer's disease via targeting endoplasmic reticulum stress from traditional chinese medicine. Comput Struct Biotechnol J 2023;23:506-519.

[46] Zhou C, Cai CP, Huang XT, et al. TarKG: a comprehensive biomedical knowledge graph for target discovery. Bioinformatics 2024;40(10):btae598.

[47] Wolfe MS, Citron M, Diehl TS, et al. A substrate-based difluoro ketone selectively inhibits Alzheimer's gamma-secretase activity. J Med Chem 1998;41(1):6-9.

[48] Yang T, Li S, Xu H, et al. Large Soluble Oligomers of Amyloid β-Protein from Alzheimer Brain Are Far Less Neuroactive Than the Smaller Oligomers to Which They Dissociate. J Neurosci 2017;37(1):152-163.

[49] Selkoe DJ. Alzheimer disease and aducanumab: adjusting our approach. Nat Rev Neurol 2019;15(7):365-366.

[50] Lane CA, Hardy J, Schott JM. Alzheimer's disease. Eur J Neurol 2018 Jan;25(1):59-70.

[51] Shannon P, Markiel A, Ozier O, el al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 2003 Nov;13(11):2498-504.

[52] Vorhees CV, Williams MT. Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nat Protoc 2006;1(2):848-58.

[53] Qi Y, Cheng X, Jing H, et al. Effect of Alpinia oxyphylla-Schisandra chinensis herb pair on inflammation and apoptosis in Alzheimer's disease mice model. J Ethnopharmacol 2019;237:28-38.

[54] Qi Y, Cheng X, Gong G, et al. Synergistic neuroprotective effect of schisandrin and nootkatone on regulating inflammation, apoptosis and autophagy via the PI3K/AKT pathway. Food Funct 2020;11(3):2427-2438.

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# Unlocking the therapeutic potential of disulfiram in sepsis: Mechanisms and future directions

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## Highlights

- Disulfiram modulates sepsis through multiple mechanisms, potentially reducing excessive inflammation and improving patient outcomes.
- The immunomodulatory effects of disulfiram are complex, influencing inflammatory mediators and immune cell function, thereby restoring immune homeostasis.
- Translating preclinical findings into clinical practice is challenging. Rigorous clinical trials and a more comprehensive understanding of the pharmacokinetics and adverse effects of disulfiram are essential.

## Abstract

The leading cause of mortality within ICUs is sepsis; however, treatment options typically fail to appropriately regulate the severely dysregulated host response to this syndrome. Recently, there has been an unexpected observation that the common alcoholic aversion treatment disulfiram has exhibited additional immune-regulatory capabilities outside of its original purpose. Disulfiram has shown some effectiveness in preclinical settings at reducing pyroptosis as well as NLRP3 inflammasome activation. Currently there is no sound systematic evidence that supports the repositioning of disulfiram for use in sepsis; more importantly, significant deficiencies in the current research were also observed and deficiencies with respect to existing investigations indicated that future studies should adopt more precise clinical approaches in order to validate the therapeutic effect of disulfiram against sepsis. Integrating the existing evidence and bringing forward feasible directions for future research also constitutes another goal of this review, which helps us better highlight the value of disulfiram as a treatment in sepsis management and further streamline research and clinical translation efforts for the development of this topic in the near future.

**Keywords:** Sepsis, disulfiram, inflammation, cell death, signaling pathways

## Introduction

The compound disulfiram was found at the end of the 19[th] century; it was originally created for industrial needs and mainly used as a vulcanization agent for rubbers. However, later on, it became known in the medical field due to its therapeutic effects [1]. The product's journey to being approved for medical treatment was rather accidental. It turned out to have some adverse reactions in the human body towards alcohol, therefore leading the FDA in the US to approve disulfiram as a solution to the problem. Alcohol dependence was moved under Disorders of Impulse Control in 1951. This transformation signified an important advancement—thanks to the unique mechanism of its action, disulfiram finally shook off the industrial chemical label that it had carried since the early days and became an actual therapeutic

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agent used in alcohol dependence treatment. Disulfiram increased bodily sensitivity to alcohol and thereby decreased drinking behaviors which proved helpful in treating individuals with alcohol dependence clinically [2].

Beyond addiction therapy, numerous diseases have been identified in which disulfiram exerts pharmacodynamic effects. These include oncological, cardiovascular, and inflammatory conditions, which have shown benefits [3, 4]. The ability of disulfiram to regulate different molecular pathways, provides a rationale for repurposing the drug for other applications [5].

There has been an increased interest among researchers regarding disulfiram as well as the fact that disulfiram might be a good candidate to participate in the drug repurposing efforts in pharmaceutical research and development. However, further studies will be conducted to further elucidate the role of disulfiram in terms of perioperative situation.

Sepsis presents an ongoing medical problem at the global level due to the underlying pathogenesis, complex mechanisms, and high mortality rate, despite significant advances in support strategies and antibiotic treatments [6]. Sepsis still represents one of the leading causes of mortality and long-term morbidity [7]. There is an immediate need to find an alternative, more effective means of treating sepsis to rescue patients from developing organ dysfunction, i. e., prolonged hypoperfusion and endotoxic shock states. Hence, repurposing some approved drugs or identifying totally new molecules has become a key part of sepsis pharmacotherapy efforts. Disulfiram can reduce inflammatory responses through multiple complex mechanisms, which has made it a promising candidate for sepsis treatment. Preclinical studies in animal models have shown that it not only lessens systemic inflammation but also alleviates organ damage and improves survival rates. Disulfiram can cut down on inflammatory reactions using many different methods, so there is some thought it might be a good choice for fighting sepsis. Results from studies using animals indicate that disulfiram can dampen down systemic inflammation, lessen organ damage, and increase survival rates compared to untreated subjects [8]. Still, most clinical trials with patients on disulfiram have focused on its safety, its effects on drug-resistant bacteria or its efficacy in treating cancer when it is given as part of another drug or intravenously [9]. These same investigators have reviewed whether disulfiram is able to modulate inflammatory cascades and other signaling pathways, as well as its current role is in managing sepsis. In addition, the review covers open questions about how this field is progressing and several points that warrant additional research.

## Pathophysiology of sepsis

Sepsis is a critical medical condition caused by the body's hyperactive immune response to infection. Sepsis causes the whole-body inflammation and organ dysfunction, and at the very worst case it even turns into shock and leads to death [5, 10]. Clinically, due to the diversity of the organ's involvement in sepsis, its manifestations include both minor physiological changes and severe multiorgan failure [11]. The connections of the immune system, inflammatory mediators, coagulation pathways, and cell dysfunction to sepsis present many challenges in diagnosing and treating this condition, with a poorer prognosis resulting from this functional disturbance [12]. The resultant organ dysfunction or even failure is a critical clinical outcome that requires prompt and aggressive interventions to prevent further deterioration of complications and patient mortality.

Sepsis is caused by a chain of inflammatory mediators and pathways that cause inflammation in the whole body and lead to dysfunction or failure of organs (**Figure 1**). Usually, sepsis occurs when the microbes are recognized based on their pathogen-associated molecular patterns (PAMPs) or endogenous damage-associated molecular patterns (DAMPs) [13, 14]. In addition, molecules of Toll-like receptors and C-type lectin are activated once PAMPs/DAMPs are recognized [15, 16]. Other pathways including nuclear factor kappa-B (NF-κB) pathway, complement system and inflammasome are subsequently activated, leading to an amplification of the damaging inflammatory cascade [16, 17].

The immune system is an important part of the development of sepsis, with its activation simultaneously acting as an aggressive and harmful mechanism. When there is an excess of inflammatory mediators, it causes hypercoagulation in the blood [18]. Mediators such as tumor necrosis factor-α, interleukin (IL)-1, and IL-6 are produced when a pathogen or toxin enters the body [19]. As the infection progresses, the immune system releases a variety of cytokines (inflammatory messengers), triggering what is known as a "cytokine storm," a phenomenon in which excessive cytokines flood the bloodstream [20]. As the disease develops, inflammation emerges as one of the immune system responses and stimulates the production of the

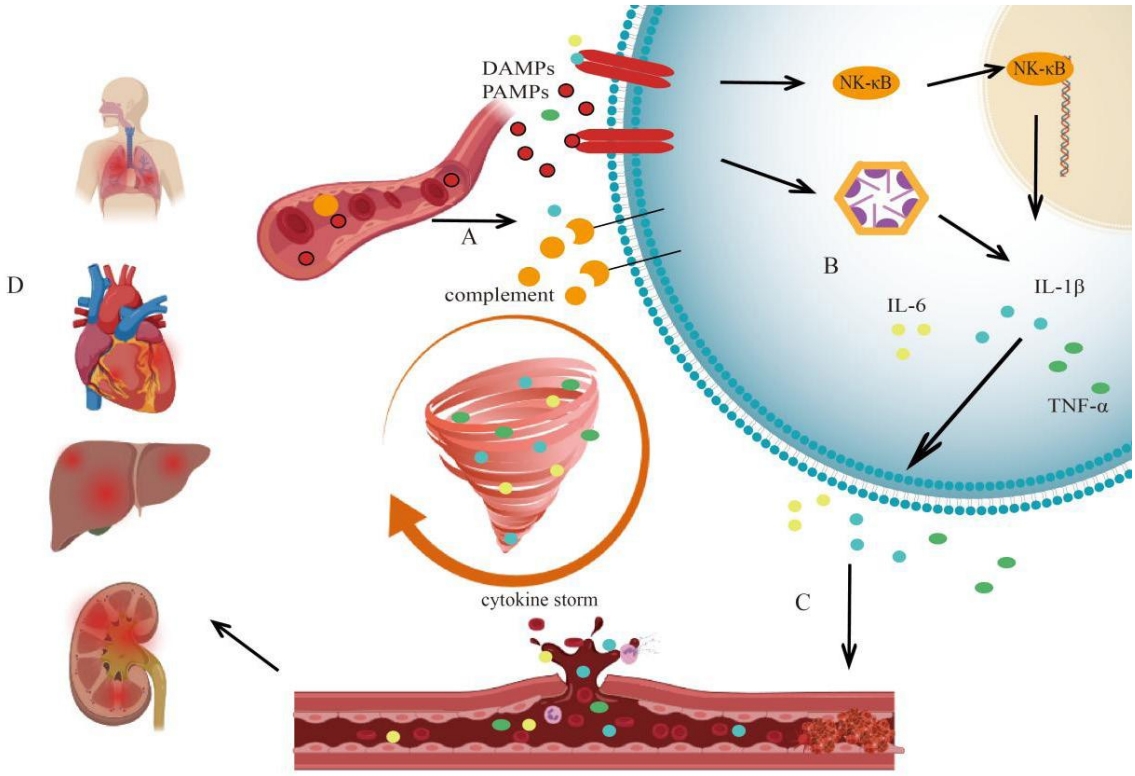


**Figure 1. The pathophysiology of sepsis.** Sepsis is triggered by a complex cascade of inflammatory mediators and associated pathways. (A) Sepsis is typically initiated by the recognition of microbial PAMPs or endogenous DAMPs, as well as complements, which activate downstream receptors, such as Toll-like receptors and C-type lectins, on the surface of cell membranes. The complement system is activated and recruits and activates immune cells; (B) The downstream inflammatory corpuscle pathway and NF-κB were activated, facilitating the release of inflammatory cytokines; (C) A substantial quantity of inflammatory cytokines is released into the bloodstream, resulting in a "cytokine storm". Tissue factor-mediated thrombin generation occurs concurrently with an imbalance or dysfunction of normal physiological anticoagulant mechanisms. Simultaneously, vascular endothelial cells are damaged, ultimately leading to systemic coagulation dysfunction, which can progress to disseminated intravascular coagulation in severe cases. Furthermore, sepsis can induce immunosuppression, diminish the immune system's resistance to infection, and increase susceptibility to secondary infection; (D) Organ oxygenation is impaired under the influence of multiple factors, such as hypotension and microthrombus formation. Endothelial cell injury further aggravates edema. Cell death intensifies, resulting in damage to multiple organ functions. PAMPs, pathogen-associated molecular patterns; DAMPs, damage-associated molecular patterns; NF-κB, nuclear factor kappa-B; TNF-α, tumor necrosis factor-α; IL, interleukin.

proteins involved in the production of clots in the body. Cytokine storms cause systemic inflammation while destroying important tissues, leading to sepsis (progression and development) [21-23]. Additionally, sepsis can lead to an immunodepression state, which suppresses the body's ability to fight infections. These opposite states of immunosuppression, resulting from hyperactivation of immune cells leading to exhaustion and apoptosis, along with an inadequate immune system, increase the risk of secondary infections and extend the disease period [24]. A compromised immune system increases susceptibility to secondary infections and can prolong the course of sepsis [25]. One of the distinctive signs of sepsis is deficient coagulation and injured vascular endothelial cells [18]. Sepsis-associated mediators increase the tissue factor expression, triggering the coagulation cascade. At the same time, the damage to the endothelial cell initiates the activation of platelets and coagulation, increasing the formation of micro-thrombus and resulting in disseminated intravascular coagulation, thus ameliorating coagulopathy is still an important challenge in sepsis research and treatment [26, 27].

Severe and complex changes take place in sepsis across multiple aspects including etiology, symptomatology, diagnosis, treatment, and prognosis, as well as through complicated and continuous dynamic mechanisms. In the initial period of sepsis, the immune system is activated instantly and powerfully with an intense inflammatory cascade and the corresponding initial immune response, involving the mobilization of multiple types of immune cells such

as neutrophils, macrophages and lymphocytes. Along with the release of large amounts of inflammatory mediators such as chemokines and prostaglandins, an acute inflammatory response occurs in sepsis, which is a defense reaction by which the body combats invading pathogens and eliminates them to maintain homeostasis. With the development of sepsis, however, the immune microenvironment gradually changes: the immune system rapidly switches from hyperactivation to suppression. Immune cells are now characterized by decreased immune function, diminished cytokine production, and increased regulatory T cell activity, among other alterations. Consequently, sepsis develops into a greater immunologic dysfunction, resulting in secondary infections and the increased severity and intricacy of the diseases [28, 29].

Therefore, a clear understanding of these dynamic, time-dependent changes in inflammatory and immune responses is critical for developing effective strategies to manage sepsis. With this knowledge, clinicians can deliver timely and targeted interventions. For example, immunomodulatory agents can be used strategically to boost immune function during the immunosuppressive phase or to reduce excessive inflammation in the early hyperinflammatory stage. By building on these insights to optimize treatment approaches, clinicians can improve patient outcomes and reduce the morbidity and mortality associated with sepsis [25].

## Historical perspective of disulfiram

### *The chemical structure and properties of disulfiram*

Disulfiram, chemically termed tetraethylthiuram disulfide and sold under the trade name Antabuse, is an organic compound with the molecular formula $C_{10}H_{20}N_2S_4$ [30]. Its core structure contains a disulfide bond that breaks down to form biologically active sulfhydryl groups [31]. At specific sites, disulfiram binds to the sulfhydryl groups of acetaldehyde dehydrogenase and oxidizes them, forming intramolecular S–S bonds. This interaction disrupts the normal function of acetaldehyde dehydrogenase, impairing its ability to convert acetaldehyde to acetic acid during ethanol metabolism in the human body [32]. As a result, acetaldehyde accumulates and leads to a variety of harmful bodily reactions, such as flushing of the face, headaches, and tachycardia. These adverse physical reactions act as impediments and discourage individuals from drinking alcohol. This antagonistic response toward alcohol can be utilized as a therapeutic strategy to encourage sobriety by fostering conditioned avoidance of alcohol among people dependent on alcohol [32, 33].

### *The metabolic process of disulfiram in the body*

The metabolic processing of disulfiram is characterized by its chelating ability, which enables it to form stable complexes with metal cations, including iron, copper, and zinc [34]. The cleavage of its disulfide bond, the primary step in its biotransformation, is central to disulfiram metabolism [31]. Upon entering the body, disulfiram undergoes reductive cleavage of its disulfide linkages, yielding thiol derivatives capable of forming mixed disulfides with endogenous protein thiols (**Figure 2**) [35]. The main metabolite, diethyldithiocarbamic (DDC) acid, is further metabolized via multiple pathways. The first pathway involves glucuronidation, in which DDC is conjugated with glucuronic acid, enhancing its hydrophilicity and promoting renal excretion [36]. The second catabolic pathway includes non-enzymatic degradation, where DDC undergoes spontaneous decomposition in the absence of enzymatic catalysis [37]. Additionally, DDC may undergo methylation and oxidation, reactions mediated by specific enzymes, further modifying the metabolite to ensure its complete catabolism and eventual elimination from the body [38-40].

### *Applications of disulfiram in medicine*

Historically used in the treatment of alcohol addiction, disulfiram has garnered increasing attention from the scientific community for its potential applications in diverse medical conditions. This shift has led to extensive research exploring the pharmacological benefits of disulfiram in various nonalcoholic pathological states [41, 42]. A substantial body of literature highlights the role of disulfiram as an aldehyde dehydrogenase inhibitor in oncological therapeutics [43, 44]. Moreover, extensive research has elucidated the promising implications of disulfiram in a wide spectrum of pathological conditions, including its ability to reduce Clostridium-mediated 7α-dehydroxylation activity in the gut microbiota, inhibit the synthesis of secondary bile acids, and potentially improve nonalcoholic steatohepatitis [45]. Disulfiram has also been shown to enhance ADAM10 activity in peripheral blood cells, alleviate A-β plaque formation, and exhibit therapeutic potential in Alzheimer's disease mouse models [46]. Furthermore, disulfiram may be useful in the treatment of obesity; however, the exact

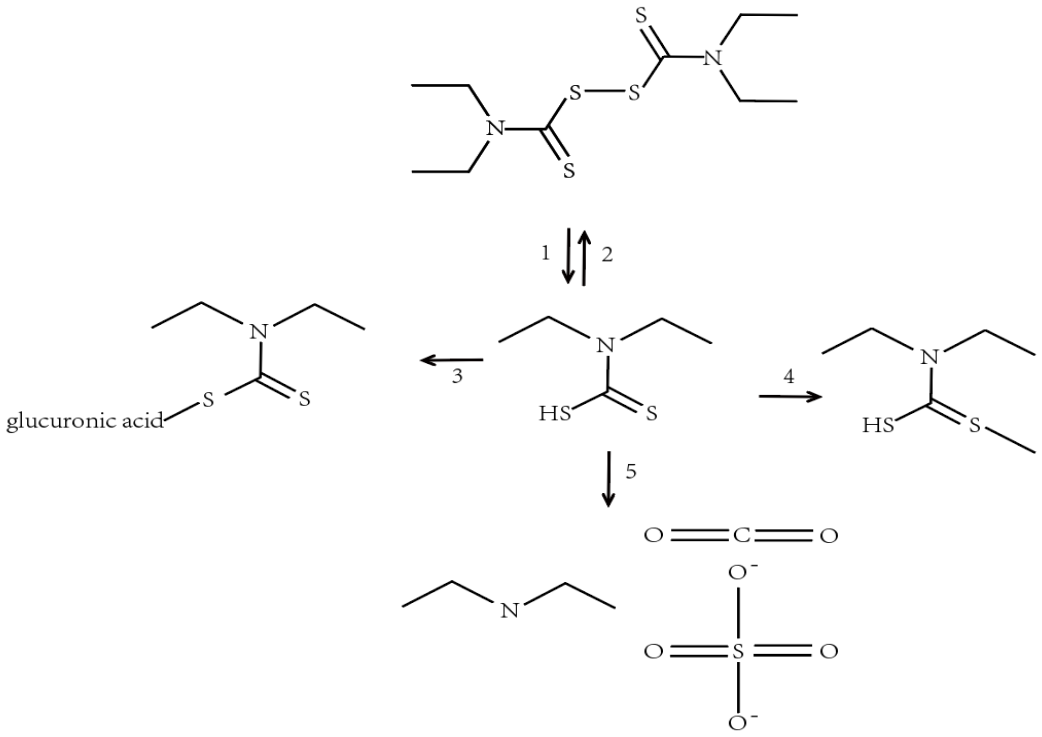


**Figure 2. Metabolic processes of disulfiram.** (1) Disulfiram undergoes cleavage to produce DDC; (2) DDC is re-oxidized to regenerate disulfiram through a reversible oxidative process; (3) DDC undergoes a conjugation reaction with glucuronic acid via the glucuronidation pathway, thereby enhancing its water solubility for subsequent excretion; (4) DDC undergoes methylation, resulting in the formation of a methyl ester derivative of DDC; (5) Disulfiram is degraded into diethylamine, carbon dioxide, and sulfate via a non-enzymatic pathway. DDC, diethyldithiocarbamate.

mechanism behind these beneficial effects remains obscure [47]. Regarding antibacterial activities, it is speculated that disulfiram can impede the growth of Pseudomonas aeruginosa via inhibiting PaBADH enzyme activity. Thus, it can suppress bacterial growth [48]. Some other studies also concluded that disulfiram might be efficacious for the treatment of Lyme disease, although the exact mechanism remains to be explored [49]. A recent report has shown that disulfiram exerts anti-sepsis effects by inhibiting macrophage pyroptosis [50]. Therefore, more and more research suggests that disulfiram is a potential therapeutic drug with pleiotropic benefits in a wide range of diseases [51]. It was suggested that its diverse impacts could result from via the modification of thiol (-SH)-containing enzymes and cofactors via different routes such as mixed disulfide formation, metal ion chelation, and non-enzymatic reactions [38]. Preclinical and early clinical results appear encouraging, but more studies are needed to validate the efficacy and safety. New uses of disulfiram present opportunities for treating a broader range of diseases. However, future studies need robust methods to achieve solid and dependable evidence; the potential adverse effects of disulfiram and the possible interaction between disulfiram and other drugs should also be thoroughly investigated. When a new clinical application of disulfiram is found, one has to evaluate whether it would benefit patients through the proper balancing of risks and benefits [51].

## The role of disulfiram in the pathophysiology of sepsis

### *The role of disulfiram in the immune response*

#### *The effects of disulfiram on the inflammatory response*

New evidence in the latter half of the twentieth century suggested that disulfiram may regulate oxidative stress and set the stage for exploring the function of disulfiram in inflammatory reactions [52-54]. Other studies have also demonstrated that disulfiram can effectively inhibit inflammatory responses [52, 55]. Lian et al. reported that local administration of disulfiram reduced psoriasis-like dermatitis in murine models [56]. Similarly, in a mouse model of Parkinson's disease, disulfiram significantly inhibited neuroinflammation and associated dopaminergic neuron death, resulting in motor function restoration [57]. In the digestive system, disulfiram inhibits colonic macrophage

activation, thereby alleviating ulcerative colitis [58]. Disulfiram also exerts immunomodulatory effects in systemic lupus erythematosus by reducing proteinuria, serum anti-dsDNA levels, and renal immune complexes, and it has been found to ameliorate crescentic glomerulonephritis [59, 60]. In the respiratory system, disulfiram has been found to attenuate lung injury in a murine model of cytomegalovirus pneumonia, leading to improved survival rates [61]. Furthermore, disulfiram mitigates vascular smooth muscle inflammation induced by angiotensin II and suppresses inflammatory responses in chondrocytes [62, 63]. These findings indicate that disulfiram may play a role in managing various inflammatory conditions. Nevertheless, more work needs to be done to clarify the underlying mechanisms and to develop it as a targeted therapy for inflammatory diseases.

*The effects of disulfiram on immune cell function*

Several studies suggest that disulfiram's impact on the immune system is likely mediated by the regulation of multiple immune cell functions. For example, research has shown that it can inhibit monocyte-to-macrophage migration, block chemotaxis, and suppress macrophage activation, which may contribute to the remission of glomerulonephritis [60]. It might also be effective in suppressing the development of macrophage pseudopods, which might hinder cell migration and invasion [64]. In fungal keratitis, disulfiram reduces the host immune response by decreasing the recruitment of macrophages and neutrophils [65].

Moreover, it regulates neutrophil extracellular traps (NETs), which are critical for neutrophil function. Studies have demonstrated that disulfiram can reduce multiorgan dysfunction and lower sepsis mortality by inhibiting NET release [8, 66]. In addition, it activates $Ca^{2+}$-ATPase of the sarcoplasmic reticulum, thereby suppressing IgE-induced eosinophil activation [67]. Furthermore, disulfiram exerts antitumor effects by acting on immune cells [68, 69]. For example, the disulfiram-copper complex can promote dendritic cell maturation, induce T cell cytotoxicity and improve antitumor immune responses [68, 70]. These findings indicate that disulfiram might be beneficial for various immune conditions, including inflammatory disorders and cancer.

***Inflammatory signaling pathways involving disulfiram***

Sepsis is triggered when microbe-derived PAMPs or endogenous DAMPs are detected by pattern recognition receptors [71]. This identification is critical for triggering subsequent signaling pathways [72]. Disulfiram, by virtue of its biochemical mechanisms, interacts with several signaling pathways (**Figure 3**). Notably, it inhibits the inflammatory response mediated by the cGAS-STING axis via RNF115. Additionally, disulfiram downregulates the complement system, a series of plasma proteins whose aberrant activation during sepsis can exacerbate tissue damage [73, 74]. At a further downstream level, disulfiram inhibits reactive oxygen species and the NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome, potentially mitigating pyroptosis, necroptosis, and mitochondrial dysfunction associated with the pathogenesis of sepsis [75, 76]. Disulfiram's interaction with autophagy, a process responsible for cellular degradation and recycling, as well as its effects on metabolic reprogramming and the coagulation cascade, suggests its potential for alleviating the cellular and systemic damage characteristic of sepsis [72]. Given the intricate network of signaling pathways involved in sepsis and their contribution to cellular and organ dysfunction, the regulatory role of disulfiram presents a promising avenue for therapeutic intervention.

*The effects of disulfiram on the NF-κB signaling pathway*

The transcription factor NF-κB is a key regulator of the inflammatory signaling pathways, particularly in sepsis [72]. PAMPs and DAMPs activate Toll-like receptor 4 on cellular membranes, initiating a cascade that ultimately activates NF-κB, leading to the induction of an inflammatory response. Recent research has highlighted the potential of disulfiram and its DSF-Cu complex to reduce the activation of NF-κB and TGF-β signaling pathways [77]. Specifically, this involves inhibiting the nuclear translocation of NF-κB subunits and promoting apoptosis in affected cells [77, 78]. Previous studies have demonstrated that disulfiram can inhibit TGF-β-induced epithelial-mesenchymal transition and the acquisition of stem-like properties in breast cancer cells via the ERK-NF-κB-Snail signaling axis [79].

While much of the current research on disulfiram's interaction with the NF-κB pathway focuses on cancer, these findings could offer valuable insights into sepsis management. Notably, disulfiram has been shown to promotes apoptosis and inhibits tumor proliferation by modulating the NF-κB signaling pathway [80, 81]. The extrapolation of these effects to the regulation

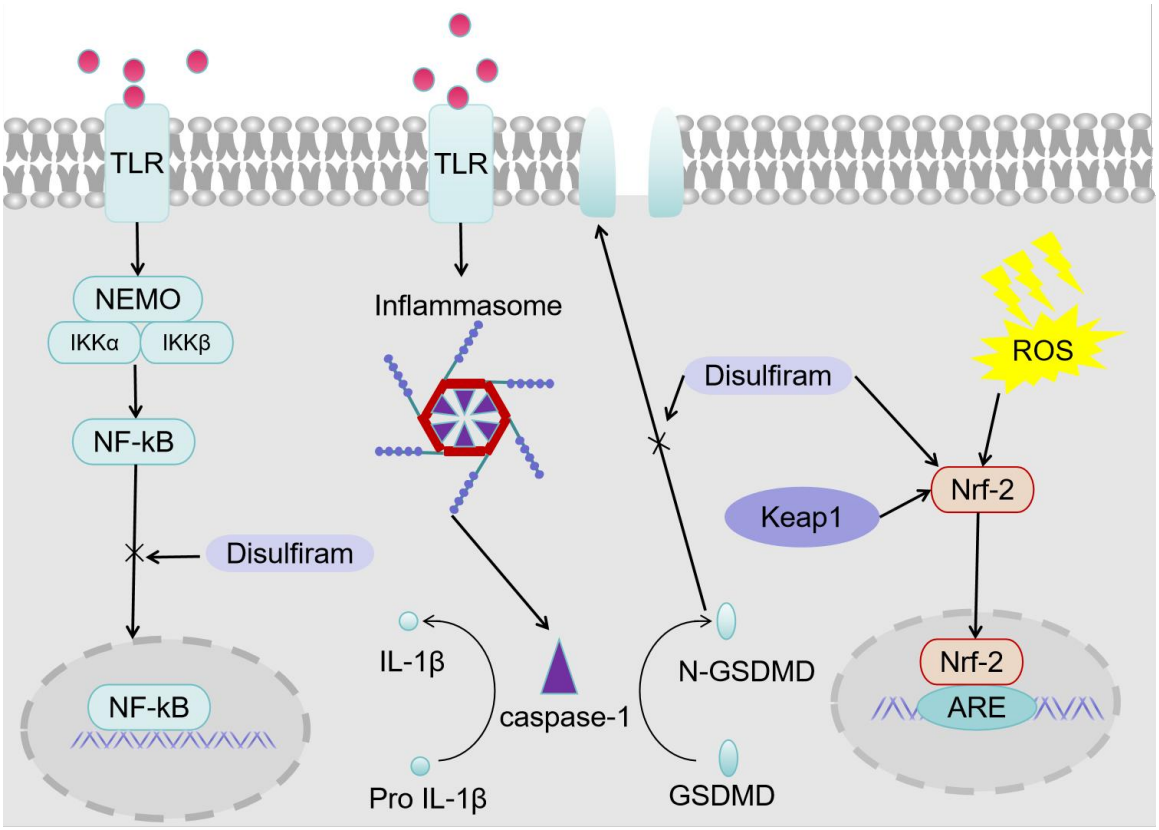


**Figure 3. The molecular pathways through which disulfiram modulates immune responses.** Disulfiram and its copper complex significantly influence cellular immune signaling by interfering with key pathways such as the NF-κB and TGF-β pathways. These compounds inhibit the nuclear translocation of NF-κB subunits and attenuate the transcriptional activity of essential inflammatory pathways. Furthermore, they induce apoptosis, contributing to their therapeutic effects in vivo. Disulfiram also inhibits the formation of gasdermin D pores in cellular membranes, which is a critical step in pyroptosis, a form of programmed cell death associated with inflammation. Moreover, the copper complex enhances the phosphorylation of p62 protein, which promotes competitive interactions with Keap1, a regulatory protein that typically mediates the degradation of Nrf2. This interaction stabilizes and prolongs the half-life of Nrf2, a master regulator of the antioxidant response, facilitating cellular defense against oxidative stress. NF-κB, nuclear factor kappa-B; IL, interleukin; TLR, toll-like-receptor; NEMO, NF-kappaB essential modulator; GSDMD, gasdermin-D; ARE, antioxidant response elements; ROS, reactive oxygen species.

of the inflammatory response in sepsis is a potentially significant area for future investigation. However, the precise mechanism by which disulfiram suppresses the NF-κB pathway in sepsis remains unclear. As most research has focused on oncology, experimental validation of the inhibitory effects of disulfiram on the NF-κB pathway in sepsis is crucial. This would not only expand the potential therapeutic strategies for sepsis but also enhance our understanding of its pathophysiology.

*The effects of disulfiram on the gasdermin D (GSDMD)-dependent pyroptotic pathway*

Pyroptosis, a highly inflammatory form of programmed cell death, plays a significant role in sepsis pathogenesis [82]. This process is initiated by the interaction of PAMPs and DAMPs with TLRs, resulting in inflammasome assembly and activation [19, 83]. The activation cascade proceeds with the proteolytic maturation of pro-caspase-1 into caspase-1, which subsequently cleaves the precursors of the proinflammatory cytokines IL-1β and IL-18, facilitating their secretion [84]. Concurrently, caspase-1 cleaves GSDMD, promoting the translocation of its N-terminal fragment to the plasma membrane. This results in the formation of pores, disruption of cellular integrity, and induction of pyroptosis [85]. In this context, disulfiram has emerged as a potential therapeutic agent capable of inhibiting GSDMD activity [86]. It binds to the Cys191 residue of human GSDMD, preventing pore formation and thereby inhibiting the cellular lysis associated with pyroptotic cell death [87]. This positions disulfiram as a promising candidate for modulating pyroptotic pathways in sepsis.

Recently, researchers found disulfiram being able to protect organs by inhibiting GSDMD especially through blocking detrimental NETs released during sepsis [8]. Studies show that

disulfiram may suppress NETs and make use of caspase-11/GSDMD pathway to alleviate ischemia reperfusion acute kidney injury [88]. Moreover, evidence suggests that disulfiram may also promote the healing of diabetic foot ulcers, indicating that its inhibition of NET formation via the NLRP3-Caspase-1-GSDMD pathway could be one of its potential therapeutic mechanisms [89]. Furthermore, these studies present multiple beneficial mechanisms of action for disulfiram in diseases involving immune abnormalities. Considering the role of disulfiram in GSDMD-mediated pyroptosis, it is worth studying and revealing the underlying mechanisms, which may provide novel targets for sepsis treatment.

*The effects of disulfiram on the nuclear factor erythroid-2 related factor 2-antioxidant response element (Nrf2-ARE) signaling pathway*

The Nrf2-ARE signaling pathway is the main defense mechanism against oxidative stress. Nrf2 is a major transcription factor in the pathway [90, 91]. When Nrf2 is exposed to oxidants, it dissociates from Keap1 that exists in the cytosol and translocates into the nucleus, and interacts with ARE. Enhancing Nrf2 activation can regulate the transcription of numerous cytoprotective genes that encode antioxidant enzymes and detoxifying proteins [92]. In sepsis, an exacerbated systemic inflammatory response results in elevated levels of reactive oxygen species and inflammatory mediators, leading to widespread tissue injury [72]. Enhancing the Nrf2-ARE pathway is crucial for cellular and tissue resilience, as it helps mitigate oxidative stress and attenuates proinflammatory cascades [93]. Recent evidence indicates that disulfiram enhances the stability and transcriptional activity of Nrf2, likely through the augmentation of p62 phosphorylation by the DSF-Cu complex. This interaction disrupts the Keap1-Nrf2 complex, stabilizing and prolonging Nrf2 activity [94]. As a result, disulfiram upregulates downstream antioxidant defense genes, creating an environment conducive to cellular protection against oxidative damage, attenuating inflammation and tissue damage induced by reactive oxygen species [94]. It's speculated that disulfiram modulates the glycogen synthase kinase-3 beta -Nrf2- NLRP3 axis, thereby inhibiting oxidative stress-induced pyroptotic cell death; by downregulating the expression of glycogen synthase kinase-3 beta and NLRP3 and upregulating the expression of Nrf2, the activity of Nrf2 is increased, oxidative stress is reduced, and immune defense is activated [95]. This indicates that disulfiram could be a potential candidate to modulate the Nrf2-ARE pathway besides treating alcohol addiction, meanwhile it is supposed that disulfiram's wide-range immunomodulatory activities surpass the boundary of the addiction therapy, and it serves as an antioxidative and anti-inflammatory agent and has been shown beneficial to intervene in oxidative stress and inflammation.

## Impact of disulfiram on sepsis progression

Sepsis is primarily treated with antibiotics to kill off infection and also by controlling any adverse inflammation response secondary to the infection. Empirical data has found that disulfiram changes immune pathways, suggesting that disulfiram may affect the pathogenesis of sepsis [74]. The primary mechanism of disulfiram involves modulation of the cellular redox state, where oxidative stress is closely linked to cellular injury and inflammation. By enhancing the Nrf2-ARE pathway, disulfiram bolsters the body's antioxidative defense, offering protection against oxidative damage [94, 95]. Furthermore, the ability of disulfiram to inhibit the NF-κB signaling cascade represents another therapeutic opportunity [96]. As NF-κB is central to the synthesis and release of pro-inflammatory mediators, its inhibition by disulfiram could significantly reduce inflammatory tissue damage [77, 78].

In addition to its immunoregulatory functions, disulfiram exhibits antimicrobial properties, although this is not its primary mechanism of action. This characteristic could complement traditional antibiotic therapy for sepsis by inhibiting the proliferation of specific pathogens, thereby aiding in the management of the infectious source [97-100]. Furthermore, experimental data highlight the efficacy of disulfiram in ameliorating cardiac dysfunction in animal models of LPS-induced cardiac injury. Disulfiram administration preserves left ventricular function and attenuates cardiomyocyte apoptosis, likely by suppressing oxidative stress and inhibiting the NLRP3 inflammasome [4, 75]. Furthermore, disulfiram can also inhibit pyroptosis by blocking the transfer of cathepsin B from lysosomes into the cytosol; it can prevent activation of the NLRP3 inflammasome as described previously. Partially due to this effect, disulfiram treatment protects against lethal sepsis through its immunomodulatory effects [101].

## Research gaps and future directions

Recent investigation shows promise for the application of disulfiram as an inducer of immunomodulatory actions especially during sepsis

[96]. Studies reveal that, in addition to lowering oxidative stress and suppressing inflammation, the compound also acts on some antimicrobial effects that might be considered as possible agents to reduce septic consequences.

However, despite these encouraging preclinical findings, a significant research gap remains in translating this knowledge into clinical practice. Although in vitro experiments and animal models offer valuable insights, they cannot fully replicate the complexities of human physiology. The discrepancy between controlled experimental conditions and the multifaceted nature of clinical sepsis, characterized by diverse etiologies and individual patient factors, poses challenges for the widespread application of disulfiram. Future research is needed to determine the feasibility of incorporating disulfiram into therapeutic regimens, particularly to clarify the precise mechanisms by which it modulates immune responses in sepsis. This immunomodulation depends on the delicate balance between proinflammatory and anti-inflammatory cytokine levels, suppression of inflammatory cascades, and preservation of immune homeostasis. Despite encouraging preclinical results, there is still a large research gap regarding the ability to translate the current knowledge of sepsis into the clinic. Although in vitro studies as well as animal models provide valuable insights, they do not adequately model all aspects of human physiology. The disparities between the controlled conditions of experimental research and the heterogeneous characteristics of clinical sepsis-stemming from its diverse etiologies and individual patient-specific factors-pose significant challenges to the broader application of disulfiram in clinical settings. More research needs to be done to see whether disulfiram could be used in the treatment protocols and to clarify the mechanisms by which disulfiram regulates the immune response, as these effects mainly result from modulating both pro- and anti-inflammatory cytokines, inhibiting pro-inflammatory cascade, and protecting immune homeostasis.

Moreover, differences between species and methodological limitations may lead to reactions that are different from human beings. In the laboratory, disulfiram concentration is much higher than the amount of human safety [102]. There are undoubtedly questions about the clinical significance of these studies. The disulfiram-ethanol reaction also poses a serious risk. Most clinical applications suffer from the severe limitation that people must totally prohibit alcohol intake and maintain a lasting state of abstinence from any kind of alcoholic beverage or product. Although disulfiram treatment can avoid the serious side effects like hepatotoxicity and peripheral neurotoxicity, rare occasions of some side effects do exist. However, up to date, the detailed molecular, cellular and systemic mechanisms behind the interaction of human immune system and disulfiram remain unclear. As such, overcoming this challenge requires extremely rigorous methods, including conducting thorough PK studies and stringent controlled trials, disulfiram has been found to interact with many other medications such that it can decrease or increase their potency or cause other adverse reactions. One lesser-studied area is the complex interaction between disulfiram and medications used to treat sepsis, including various antibiotics (β-lactams, fluoroquinolones, glycopeptides, etc.) and vasoactive agents (norepinephrine, vasopressin). Disulfiram irreversibly inhibits aldehyde dehydrogenase and cytochrome P450 (CYP) enzymes, notably CYP2E1 and CYP3A4, which are key for the metabolism of many antibiotics and vasoactive agents [103]. The antioxidant effects of disulfiram may counteract the pro-oxidant mechanisms of certain antibiotics, thereby reducing their bactericidal efficacy. Sepsis-induced hepatic and renal impairments alter the pharmacokinetics of disulfiram; however, no study has characterized dose adjustments in this context.

Future studies should focus on the complexities and potential of disulfiram as a therapeutic agent for sepsis. These investigations should elucidate the dose-response relationship, therapeutic potential, and safety profile of disulfiram. Understanding the pharmacokinetic profile, possible adverse effects, and interactions with existing therapeutic regimens is thus crucial. As a result, encouraging findings from initial studies can lead to improved phases of clinical trials that may radically change the way sepsis is treated.

With further advances in research, efforts are underway to integrate biomarker analysis for predicting therapeutic effects and to develop personalized treatment regimens, which can not only boost our recognition of the role played by disulfiram in sepsis but also enable translational application of the agent so as to attain best possible treatment strategy based on individual needs.

## Conclusion

In summary, further investigation of disulfiram's immunomodulatory effects, as well as its potential application to sepsis therapy could open

up new doors in biomedicine given that some initial pre-clinical evidence appears to indicate a possible benefit in this area. Additional work must focus on the immunological effects of this molecule and establish whether it offers therapeutic value against sepsis; more fundamental work is need to clarify the precise mechanisms through which disulfiram acts upon the immune system and inflammatory pathways in order to utilise it effectively as an immunomodulator for targeted therapeutic interventions; and ideally, thorough clinical trials would confirm whether disulfiram has therapeutic value in the setting of sepsis and demonstrate an acceptable safety profile.

The potential value of applying disulfiram as a novel therapeutic agent for the treatment of sepsis cannot be understated; however, caution is warranted in the pursuit of this goal, with a full appreciation of the scientific rationale guiding the potential immunomodulatory mechanisms to which it may contribute. It is only via robust research studies followed by meticulous clinical trials that the full therapeutic scope of using disulfiram in sepsis and other severe infection states will be defined.

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## References

[1] Dosset M, Zanetti M. Disulfiram's journey from rubber vulcanization to T-cell activation. Embo j 2022;41(16):e111862.

[2] Hald J, Jacobsen E. A drug sensitizing the organism to ethyl alcohol. Lancet 1948;2(6539):1001-1004.

[3] Li Q, Chao Y, Liu B, et al. Disulfiram loaded calcium phosphate nanoparticles for enhanced cancer immunotherapy. Biomaterials 2022;291:121880.

[4] Wei S, Xiao Z, Huang J, et al. Disulfiram inhibits oxidative stress and NLRP3 inflammasome activation to prevent LPS-induced cardiac injury. Int Immunopharmacol 2022;105:108545.

[5] Angus DC, van der Poll T. Severe sepsis and septic shock. N Engl J Med 2013;369(9):840-851.

[6] Rudd KE, Johnson SC, Agesa KM, et al. Global, regional, and national sepsis incidence and mortality, 1990-2017: analysis for the Global Burden of Disease Study. Lancet 2020;395(10219):200-211.

[7] Cecconi M, Evans L, Levy M, et al. Sepsis and septic shock. Lancet 2018;392(10141):75-87.

[8] Silva CMS, Wanderley CWS, Veras FP, et al. Gasdermin D inhibition prevents multiple organ dysfunction during sepsis by blocking NET formation. Blood 2021;138(25):2702-2713.

[9] Nechushtan H, Hamamreh Y, Nidal S, et al. A phase IIb trial assessing the addition of disulfiram to chemotherapy for the treatment of metastatic non-small cell lung cancer. Oncologist 2015;20(4):366-367.

[10] Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). Jama 2016;315(8):801-810.

[11] Jacobi J. The pathophysiology of sepsis - 2021 update: Part 2, organ dysfunction and assessment. Am J Health Syst Pharm 2022;79(6):424-436.

[12] De Backer D, Deutschman CS, Hellman J, et al. Surviving Sepsis Campaign Research Priorities 2023. Crit Care Med 2024;52(2):268-296.

[13] Cicchinelli S, Pignataro G, Gemma S, et al. PAMPs and DAMPs in Sepsis: A Review of Their Molecular Features and Potential Clinical Implications. Int J Mol Sci 2024;25(2):962.

[14] Zindel J, Kubes P. DAMPs, PAMPs, and LAMPs in Immunity and Sterile Inflammation. Annu Rev Pathol 2020;15:493-518.

[15] Nofi CP, Wang P, Aziz M. Chromatin-Associated Molecular Patterns (CAMPs) in sepsis. Cell Death Dis 2022;13(8):700.

[16] Zhang YY, Ning BT. Signaling pathways and intervention therapies in sepsis. Signal Transduct Target Ther 2021;6(1):407.

[17] Guo Q, Jin Y, Chen X, et al. NF-κB in biology and targeted therapy: new insights and translational implications. Signal Transduct Target Ther 2024;9(1):53.

[18] Tsantes AG, Parastatidou S, Tsantes EA, et al. Sepsis-Induced Coagulopathy: An Update on Pathophysiology, Biomarkers, and Current Guidelines. Life (Basel) 2023;13(2):350.

[19] Zheng D, Liwinski T, Elinav E. Inflammasome activation and regulation: toward a better understanding of complex mechanisms. Cell Discov 2020;6:36.

[20] Jarczak D, Nierhaus A. Cytokine Storm-Definition, Causes, and Implications. Int J Mol Sci 2022;23(19):11740.

[21] Remick DG. Pathophysiology of sepsis. Am J Pathol 2007;170(5):1435-1444.

[22] Karki R, Sharma BR, Tuladhar S, et al. Synergism of TNF-α and IFN-γ Triggers Inflammatory Cell Death, Tissue Damage, and Mortality in SARS-CoV-2 Infection and Cytokine Shock Syndromes. Cell 2021;184(1):149-168.e117.

[23] Chousterman BG, Swirski FK, Weber GF. Cytokine storm and sepsis disease pathogenesis.

Semin Immunopathol 2017;39(5):517-528.
[24] Cao M, Wang G, Xie J. Immune dysregulation in sepsis: experiences, lessons and perspectives. Cell Death Discov 2023;9(1):465.
[25] Liu D, Huang SY, Sun JH, et al. Sepsis-induced immunosuppression: mechanisms, diagnosis and current treatment options. Mil Med Res 2022;9(1):56.
[26] Levi M, van der Poll T. Inflammation and coagulation. Crit Care Med 2010;38:2 Suppl):S26-S34.
[27] Levi M, Keller TT, van Gorp E, et al. Infection and inflammation and the coagulation system. Cardiovasc Res 2003;60(1):26-39.
[28] Giamarellos-Bourboulis EJ, Aschenbrenner AC, Bauer M, et al. The pathophysiology of sepsis and precision-medicine-based immunotherapy. Nat Immunol 2024;25(1):19-28.
[29] Cajander S, Kox M, Scicluna BP, et al. Profiling the dysregulated immune response in sepsis: overcoming challenges to achieve the goal of precision medicine. Lancet Respir Med 2024;12(4):305-322.
[30] The chemical structural formula of disulfiram. Available at: http://www.chemspider.com/Chemical-Structure.3005.html?rid=3d01c36d-efa7-4189-8e2a-78eb54dcf890
[31] Kjeldgaard NO. Inhibition of Aldehyde Oxidase from Liver by Tetraethylthiuramdisulphide (Antabuse). Acta Pharmacol Toxicol 1949;5(4):397-403.
[32] Vallari RC, Pietruszko R. Human aldehyde dehydrogenase: mechanism of inhibition of disulfiram. Science 1982;216(4546):637-639.
[33] Suh JJ, Pettinati HM, Kampman KM, et al. The status of disulfiram: a half of a century later. J Clin Psychopharmacol 2006;26(3):290-302.
[34] Eneanya DI, Bianchine JR, Duran DO, et al. The Actions and Metabolic Fate of Disulfiram. Pharmacol Toxicol 1981;21(1):575-596.
[35] Nagy P. Kinetics and Mechanisms of Thiol–Disulfide Exchange Covering Direct Substitution and Thiol Oxidation-Mediated Pathways. Antioxid Redox Signal 2013;18(13):1623-1641.
[36] Faiman MD, Jensen JC, Lacoursiere RB. Elimination kinetics of disulfiram in alcoholics after single and repeated doses. Clin Pharmacol Ther 1984;36(4):520-526.
[37] Nagendra SN, Shetty KT, Subhash MN, et al. Role of glutathione reductase system in disulfiram conversion to diethyldithiocarbamate. Life Sci 1991;49(1):23-28.
[38] Eneanya DI, Bianchine JR, Duran DO, et al. The actions of metabolic fate of disulfiram. Annu Rev Pharmacol Toxicol 1981;21:575-596.
[39] Yourick JJ, Faiman MD. Comparative aspects of disulfiram and its metabolites in the disulfiram-ethanol reaction in the rat. Biochem Pharmacol 1989;38(3):413-421.
[40] Palanski BA, Khosla C. Cystamine and Disulfiram Inhibit Human Transglutaminase 2 via an Oxidative Mechanism. Biochemistry 2018;57(24):3359-3363.
[41] Farooq MA, Aquib M, Khan DH, et al. Recent advances in the delivery of disulfiram: a critical analysis of promising approaches to improve its pharmacokinetic profile and anticancer efficacy. Daru 2019;27(2):853-862.
[42] Guo W, Chen S, Li C, et al. Application of Disulfiram and its Metabolites in Treatment of Inflammatory Disorders. Front Pharmacol 2021;12:795078.
[43] Zhang G, Wang Y, Fuchs BC, et al. Improving the Therapeutic Efficacy of Sorafenib for Hepatocellular Carcinoma by Repurposing Disulfiram. Front Oncol 2022;12:913736.
[44] Ni YL, Chien PJ, Hsieh HC, et al. Disulfiram/Copper Suppresses Cancer Stem Cell Activity in Differentiated Thyroid Cancer Cells by Inhibiting BMI1 Expression. Int J Mol Sci 2022;23(21):13276.
[45] Lei Y, Tang L, Chen Q, et al. Disulfiram ameliorates nonalcoholic steatohepatitis by modulating the gut microbiota and bile acid metabolism. Nat Commun 2022;13(1):6862.
[46] Reinhardt S, Stoye N, Luderer M, et al. Identification of disulfiram as a secretase-modulating compound with beneficial effects on Alzheimer's disease hallmarks. Sci Rep 2018;8(1):1329.
[47] Bernier M, Mitchell SJ, Wahl D, et al. Disulfiram Treatment Normalizes Body Weight in Obese Mice. Cell Metab 2020;32(2):203-214.e204.
[48] Zaldívar-Machorro VJ, López-Ortiz M, Demare P, et al. The disulfiram metabolites S-methyl-N,N-diethyldithiocarbamoyl sulfoxide and S-methyl-N,N-diethylthiocarbamoyl sulfone irreversibly inactivate betaine aldehyde dehydrogenase from Pseudomonas aeruginosa, both in vitro and in situ, and arrest bacterial growth. Biochimie 2011;93(2):286-295.
[49] Gao J, Gong Z, Montesano D, et al. "Repurposing" Disulfiram in the Treatment of Lyme Disease and Babesiosis: Retrospective Review of First 3 Years' Experience in One Medical Practice. Antibiotics (Basel) 2020;9(12):868.
[50] Ou AT, Zhang JX, Fang YF, et al. Disulfiram-loaded lactoferrin nanoparticles for treating inflammatory diseases. Acta Pharmacol Sin 2021;42(11):1913-1920.
[51] Cvek B. The Promiscuity of Disulfiram in Medicinal Research. ACS Med Chem Lett 2023;14(12):1610-1614.
[52] Ningaraj NS, Rao MK. Disulfiram augments oxidative stress in rat brain following bilateral carotid artery occlusion. J Biomed Sci 1998;5(3):226-230.
[53] Delmaestro E, Trombetta LD. The effects of di-

sulfiram on the hippocampus and cerebellum of the rat brain: a study on oxidative stress. Toxicol Lett 1995;75(1-3):235-243.
[54] Kyle ME, Serroni A, Farber JL. The inhibition of lipid peroxidation by disulfiram prevents the killing of cultured hepatocytes by allyl alcohol, tert-butyl hydroperoxide, hydrogen peroxide and diethyl maleate. Chem Biol Interact 1989;72(3):269-275.
[55] Forman HJ, York JL, Fisher AB. Mechanism for the potentiation of oxygen toxicity by disulfiram. J Pharmacol Exp Ther 1980;212(3):452-455.
[56] Lian N, Chen Y, Chen S, et al. Gasdermin D-mediated keratinocyte pyroptosis as a key step in psoriasis pathogenesis. Cell Death Dis 2023;14(9):595.
[57] Bai Y, Min R, Chen P, et al. Disulfiram blocks inflammatory TLR4 signaling by targeting MD-2. Proc Natl Acad Sci U S A 2023;120(31):e2306399120.
[58] Zhou W, Zhang H, Huang L, et al. Disulfiram with Cu(2+) alleviates dextran sulfate sodium-induced ulcerative colitis in mice. Theranostics 2023;13(9):2879-2895.
[59] Zhuang L, Luo X, Wu S, et al. Disulfiram alleviates pristane-induced lupus via inhibiting GSDMD-mediated pyroptosis. Cell Death Discov 2022;8(1):379.
[60] Toda E, Sawada A, Takeuchi K, et al. Inhibition of the chemokine signal regulator FROUNT by disulfiram ameliorates crescentic glomerulonephritis. Kidney Int 2022;102(6):1276-1290.
[61] Huang X, Sun P, Qin Y, et al. Disulfiram attenuates MCMV-Induced pneumonia by inhibition of NF-κB/NLRP3 signaling pathway in immunocompromised mice. Int Immunopharmacol 2022;103:108453.
[62] Liao F, Wang L, Wu Z, et al. Disulfiram protects against abdominal aortic aneurysm by ameliorating vascular smooth muscle cells pyroptosis. Cardiovasc Drugs Ther 2023;37(6):1-14.
[63] Li C, Li L, Lan T. Co-treatment with disulfiram and glycyrrhizic acid suppresses the inflammatory response of chondrocytes. J Orthop Surg Res 2021;16(1):132.
[64] Ikebukuro T, Arima T, Kasamatsu M, et al. Disulfiram Ophthalmic Solution Inhibited Macrophage Infiltration by Suppressing Macrophage Pseudopodia Formation in a Rat Corneal Alkali Burn Model. Int J Mol Sci 2023;24(1):735.
[65] Yan H, Yang H, Wang L, et al. Disulfiram inhibits IL-1β secretion and inflammatory cells recruitment in Aspergillus fumigatus keratitis. Int Immunopharmacol 2022;102:108401.
[66] Silva CMS, Wanderley CWS, Veras FP, et al. Gasdermin-D activation by SARS-CoV-2 triggers NET and mediate COVID-19 immunopathology. Crit Care 2022;26(1):206.
[67] Selnø ATH, Sumbayev VV, Gibbs BF. IgE-dependent human basophil responses are inversely associated with the sarcoplasmic reticulum Ca(2+)-ATPase (SERCA). Front Immunol 2022;13:1052290.
[68] Wang Q, Zhu T, Miao N, et al. Disulfiram bolsters T-cell anti-tumor immunity through direct activation of LCK-mediated TCR signaling. Embo j 2022;41(16):e110636.
[69] Zhang S, Zong Y, Chen L, et al. The immunomodulatory function and antitumor effect of disulfiram: paving the way for novel cancer therapeutics. Discov Oncol 2023;14(1):103.
[70] Gao X, Huang H, Pan C, et al. Disulfiram/Copper Induces Immunogenic Cell Death and Enhances CD47 Blockade in Hepatocellular Carcinoma. Cancers (Basel) 2022;14(19):4175.
[71] Rajaee A, Barnett R, Cheadle WG. Pathogen- and Danger-Associated Molecular Patterns and the Cytokine Response in Sepsis. Surg Infect (Larchmt) 2018;19(2):107-116.
[72] Zhang Y-y, Ning B-t. Signaling pathways and intervention therapies in sepsis. Signal Transduct Target Ther 2021;6(1):407.
[73] Adrover JM, Carrau L, Daßler-Plenker J, et al. Disulfiram inhibits neutrophil extracellular trap formation and protects rodents from acute lung injury and SARS-CoV-2 infection. JCI Insight 2022;7(5):e157342.
[74] Zhang ZD, Shi CR, Li FX, et al. Disulfiram ameliorates STING/MITA-dependent inflammation and autoimmunity by targeting RNF115. Cell Mol Immunol 2024;21(3):275-291.
[75] Deng W, Yang Z, Yue H, et al. Disulfiram suppresses NLRP3 inflammasome activation to treat peritoneal and gouty inflammation. Free Radic Biol Med 2020;152:8-17.
[76] Zhang X, Wang Z, Zheng Y, et al. Inhibitors of the NLRP3 inflammasome pathway as promising therapeutic candidates for inflammatory diseases (Review). Int J Mol Med 2023;51(4):35.
[77] Li Y, Wang LH, Zhang HT, et al. Disulfiram combined with copper inhibits metastasis and epithelial-mesenchymal transition in hepatocellular carcinoma through the NF-κB and TGF-β pathways. J Cell Mol Med 2018;22(1):439-451.
[78] Zhu Y, Lei C, Jiang Q, et al. DSF/Cu induces antitumor effect against diffuse large B-cell lymphoma through suppressing NF-κB/BCL6 pathways. Cancer Cell Int 2022;22(1):236.
[79] Han D, Wu G, Chang C, et al. Disulfiram inhibits TGF-β-induced epithelial-mesenchymal transition and stem-like features in breast cancer via ERK/NF-κB/Snail pathway. Oncotarget 2015;6(38):40907-40919.
[80] Hu JJ, Liu X, Xia S, et al. FDA-approved disulfiram inhibits pyroptosis by blocking gasdermin D pore formation. Nat Immunol 2020;21(7):736-745.

[81] Wang L, Yu Y, Zhou C, et al. Anticancer effects of disulfiram: a systematic review of in vitro, animal, and human studies. Syst Rev 2022;11(1):109.
[82] Wei X, Xie F, Zhou X, et al. Role of pyroptosis in inflammation and cancer. Cell Mol Immunol 2022;19(9):971-992.
[83] Yao J, Sterling K, Wang Z, et al. The role of inflammasomes in human diseases and their potential as therapeutic targets. Signal Transduct Target Ther 2024;9(1):10.
[84] Bertheloot D, Latz E, Franklin BS. Necroptosis, pyroptosis and apoptosis: an intricate game of cell death. Cell Mol Immunol 2021;18(5):1106-1121.
[85] Devant P, Kagan JC. Molecular mechanisms of gasdermin D pore-forming activity. Nat Immunol 2023;24(7):1064-1075.
[86] Nie D, Chen C, Li Y, et al. Disulfiram, an aldehyde dehydrogenase inhibitor, works as a potent drug against sepsis and cancer via NETosis, pyroptosis, apoptosis, ferroptosis, and cuproptosis. Blood Sci 2022;4(3):152-154.
[87] Hu JJ, Liu X, Xia S, et al. FDA-approved disulfiram inhibits pyroptosis by blocking gasdermin D pore formation. Nat Immunol 2020;21(7):736-745.
[88] Cai Q, Sun Z, Xu S, et al. Disulfiram ameliorates ischemia/reperfusion-induced acute kidney injury by suppressing the caspase-11-GSDMD pathway. Ren Fail 2022;44(1):1169-1181.
[89] Yang S, Feng Y, Chen L, et al. Disulfiram accelerates diabetic foot ulcer healing by blocking NET formation via suppressing the NLRP3/Caspase-1/GSDMD pathway. Transl Res 2023;254:115-127.
[90] Nguyen T, Nioi P, Pickett CB. The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress. J Biol Chem 2009;284(20):13291-13295.
[91] Wu X, Wei J, Yi Y, et al. Activation of Nrf2 signaling: A key molecular mechanism of protection against cardiovascular diseases by natural products. Front Pharmacol 2022;13:1057918.
[92] Lu MC, Ji JA, Jiang ZY, et al. The Keap1-Nrf2-ARE Pathway As a Potential Preventive and Therapeutic Target: An Update. Med Res Rev 2016;36(5):924-963.
[93] Ngo V, Duennwald ML. Nrf2 and Oxidative Stress: A General Overview of Mechanisms and Implications in Human Disease. Antioxidants (Basel) 2022;11(12):2345.
[94] Ren X, Li Y, Zhou Y, et al. Overcoming the compensatory elevation of NRF2 renders hepatocellular carcinoma cells more vulnerable to disulfiram/copper-induced ferroptosis. Redox Biol 2021;46:102122.
[95] Chi F, Zhang G, Ren N, et al. The anti-alcoholism drug disulfiram effectively ameliorates ulcerative colitis through suppressing oxidative stresses-associated pyroptotic cell death and cellular inflammation in colonic cells. Int Immunopharmacol 2022;111:109117.
[96] Wang W, McLeod HL, Cassidy J. Disulfiram-mediated inhibition of NF-kappaB activity enhances cytotoxicity of 5-fluorouracil in human colorectal cancer cell lines. Int J Cancer 2003;104(4):504-511.
[97] Long TE. Repurposing Thiram and Disulfiram as Antibacterial Agents for Multidrug-Resistant Staphylococcus aureus Infections. Antimicrob Agents Chemother 2017;61(9):e00898-17.
[98] Huang W, Zhang J, Liu S, et al. Disulfiram Enhances the Activity of Polymyxin B Against Klebsiella pneumoniae by Inhibiting Lipid A Modification. Infect Drug Resist 2022;15:295-306.
[99] Horita Y, Takii T, Yagi T, et al. Antitubercular activity of disulfiram, an antialcoholism drug, against multidrug- and extensively drug-resistant Mycobacterium tuberculosis isolates. Antimicrob Agents Chemother 2012;56(8):4140-4145.
[100] Lanz J, Biniaz-Harris N, Kuvaldina M, et al. Disulfiram: Mechanisms, Applications, and Challenges. Antibiotics 2023;12(3):524.
[101] Liu C, Tang J, Liu S, et al. Cathepsin B/NLRP3/GSDMD axis-mediated macrophage pyroptosis induces inflammation and fibrosis in systemic sclerosis. J Dermatol Sci 2022;108(3):127-137.
[102] Lanz J, Biniaz-Harris N, Kuvaldina M, et al. Disulfiram: Mechanisms, Applications, and Challenges. Antibiotics (Basel) 2023;12(3):524.
[103] Madan A, Parkinson A, Faiman MD. Identification of the human and rat P450 enzymes responsible for the sulfoxidation of S-methyl N,N-diethylthiolcarbamate (DETC-ME). The terminal step in the bioactivation of disulfiram. Drug Metab Dispos 1995;23(10):1153-1162.

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# Research progress on ferroptosis in the pathogenesis of sepsis

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## Highlights

- Lipid metabolism and iron metabolism pathways are key biological pathways involved in ferroptosis.
- Ferroptosis exhibits complex crosstalk with other cell death forms in sepsis, with significant implications for disease pathogenesis and therapy.
- Ferroptosis plays a critical role in sepsis-related organ damage and prognosis.
- This review suggests directions and perspectives for treating ferroptosis and sepsis in the perioperative period.

## Abstract

Ferroptosis is a newly identified form of cell death that has garnered attention in recent years. Current research has clarified several key mechanisms of ferroptosis, with reactive oxygen species, oxidative stress, and iron metabolism emerging as central factors. Additionally, various signaling pathways, molecules, and organelles also contribute to the regulation and progression of ferroptosis. Activation of ferroptosis has significant implications for sepsis-related inflammation, providing both a valuable area for scientific investigation and a potential target for therapeutic interventions. This article reviews the biological processes and molecular mechanisms of ferroptosis, as well as the small molecules and signaling pathways that regulate it. Additionally, we discuss the role of ferroptosis in the progression of sepsis and its contribution to organ damage.

**Keywords:** Ferroptosis, sepsis, inflammation, GPX4

## I Introduction

Sepsis is a systemic inflammatory response syndrome caused by the invasion of pathogenic microorganisms into the body [1, 2]. Based on global research data, it is estimated that sepsis incidence exceeds 48.9 million new cases annually, with approximately 11 million sepsis-related deaths, accounting for 19.7% of all global mortalities [3]. This imposes a substantial burden on healthcare systems and represents a significant threat to human health. Recent global estimates derived from data on hospitalized adults in seven high-income countries report an annual incidence of 19.4 million sepsis cases (formerly severe sepsis) and 5.3 million sepsis-related deaths [3]. These figures suggest that sepsis may be more prevalent and lethal than currently estimated, warranting further research and attention [4].

However, therapeutic advances in sepsis remain largely limited to supportive care, including organ support and fluid resuscitation [5, 6]. There remains a gap in the development of effective medications for sepsis, making it crucial to explore new therapeutic directions and pathophysiological mechanisms [7].

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Recently, ferroptosis, a novel form of regulated cell death, has been implicated in sepsis, although its precise relationship remains incompletely understood. Sepsis has been described as a failed starvation response, where the body undergoes an intense starvation reaction, involving the production of high-energy metabolites such as lactate and free fatty acids [8]. Investigating the mechanisms of oxidative stress, ferroptosis, and other metabolic abnormalities in sepsis, particularly those related to lipid peroxidation and cellular ion metabolism, may provide new insights and potential targets for sepsis management.

## II Ferroptosis: A novel form of regulated cell death

In 2012, Professor Stockwell identified a molecule, Erastin, that activates iron-dependent cell death, which was subsequently named ferroptosis [9]. Ferroptosis represents a novel regulatory form of cell death distinct from other forms, such as apoptosis, due to its unique morphological, biochemical, or genetic characteristics. Morphologically, ferroptosis is typically characterized by a reduction in mitochondrial volume, increased mitochondrial membrane density, and a loss or disappearance of mitochondrial cristae, while the cell membrane, nucleus, and chromatin remain largely unaffected [10]. Biochemically, ferroptosis involves the depletion of intracellular nicotinamide adenine dinucleotide hydride and glutathione (GSH), a reduction in the activity of glutathione peroxidase 4 (GPX4), and an accumulation of reactive oxygen species (ROS), all of which promote ferroptosis. Genetically, various cancer-related genes have been shown to influence cellular sensitivity to ferroptosis [11].

### Biological processes associated with ferroptosis

Ferroptosis is characterized by iron ion-mediated oxidative damage. An increase in iron-dependent ROS within cells, inhibition of GPX4, or depletion of GSH can all trigger ferroptosis. Recent research has identified three distinct pathways involved in ferroptosis: the classical GPX4 regulatory pathway, the lipid metabolism pathway, and the iron metabolism pathway [12].

#### *The classical GPX4 regulatory pathway*

Erastin, a small molecule compound, selectively induces cell death in tumor cells expressing Small T oncoprotein (ST) and RAS by activating ferroptosis. It operates through various molecular mechanisms, including the cystine-glutamate transporter (system $Xc^-$), voltage-dependent anion channels, and p53. Erastin inhibits the cystine-glutamate antiporter system, leading to a depletion of intracellular cystine in the cell, which in turn disrupts the normal synthesis of GSH and inactivates intracellular GPX4. Both GPX4 and GSH are essential for cellular antioxidant defense, and their inactivation leads to the accumulation of lipid ROS and other peroxides, damaging proteins or membranes, ultimately inducing ferroptosis [13, 14].

#### *Lipid metabolism pathways*

Lipid peroxidation is a hallmark of ferroptosis, with lipid peroxides playing a crucial role in this process [15]. Polyunsaturated fatty acids increase membrane fluidity, which are crucial for cellular adaptation, but also serve as substrates for lipoxygenases, which catalyze the formation of pro-ferroptotic lipid peroxides [16]. Arachidonic acid and adrenic acid are two primary polyunsaturated fatty acids involved in lipid peroxidation during ferroptosis. Synthetase long chain family member 4 (Acyl-CoA) synthetase long-chain family member 4 (ACSL4) facilitates the conjugation of unsaturated fatty acids with coenzyme A, forming acyl-CoA [17, 18]. Subsequently, acyl-CoA can undergo re-acylation through various lysolipid phosphatidylcholine acyltransferases, with ACSL4 and lysolipid phosphatidylcholine acyltransferases 3 playing an essential role in the biosynthesis and remodeling of arachidonic acid/adrenic acid derivatives. Lipid peroxides themselves generate phospholipid hydroperoxides, which degrade into malondialdehyde (MDA) and 4-hydroxynonenal. These products induce cytotoxicity, destabilizing cell membranes and leading to cell death [19, 20].

#### *Iron metabolism pathway*

Although iron is a trace element, it plays a vital role in cellular metabolism. It is absorbed as non-heme iron in the small intestine, binds to transferrin in the serum, and is subsequently recognized by the transferrin receptor on the cell membrane. Once internalized by the transferrin receptor, STEAP3 metalloreductase reduces trivalent iron ions to divalent iron ions, which are then stored in the labile iron pool and ferritin [21]. Ferritin, composed of the ferritin light chain and ferritin heavy chain 1, can be degraded by lysosomes to increase free iron [22]. Under normal circumstances, excess divalent iron is oxidized to trivalent iron to iron homeostasis [23]. However, when iron homeostasis is disrupted, excess iron can act as a

cofactor in oxidative stress, promoting erastin-induced ferroptosis by depleting cysteine desulfurase (NFS1), thereby inhibiting iron-sulfur cluster biosynthesis [24].

### Signaling pathways related to ferroptosis

The modulation of ferroptosis-associated processes, such as molecular regulation, lipid metabolism, oxidative stress, and iron metabolism, can influence ferroptosis. Research has shown that the oxidative stress transcription factor nuclear factor erythroid 2-related factor 2 (NRF2) can stimulate the expression of multiple target genes to mitigate ferroptosis [25]. Furthermore, accumulating evidence suggests that several signaling pathways determine a cell's susceptibility to ferroptosis within specific biological contexts. These signaling pathways can be broadly categorized into GPX4-dependent pathways (for instance, the classical system $Xc^-$: GSH-GPX4 axis) and GPX4-independent pathways [26].

The system $Xc^-$, specifically the GSH–GPX4 axis, is an amino acid antiporter composed of two essential subunits: solute carrier family 7 member 11 (SLC7A11/xCT) and SLC3A2 [27]. This system typically facilitates the exchange of extracellular cystine and intracellular glutamate across the plasma membrane. The expression or activity of SLC7A11 can be finely tuned through protein-protein interactions (e.g., BECN1), gene transcription (e.g., *NFE2L2* or *TP53*), or protein degradation (e.g., via OTU deubiquitinating enzymes like OTUB1). Once cystine enters the cell, it is reduced to cysteine, which is subsequently utilized for the synthesis of GSH. Glutathione, also known as L- $\gamma$ -Glutamyl-L-cysteinyl-glycine, is a tripeptide composed of glutamate, cysteine, and glycine, and plays a critical role in cellular antioxidant defense [28]. The reactive thiol group (-SH) of GSH is readily oxidizable. During glutathione synthesis, $\gamma$ -glutamylcysteine synthetase ( $\gamma$ -GCS) catalyzes the reaction between glutamate and cysteine to produce gamma-glutamylcysteine [29]. As a potent reducing agent, glutathione effectively converts harmful lipid hydroperoxides generated during cellular metabolism into harmless lipid alcohols, a process that is crucial for determining cellular susceptibility to ferroptosis [30].

GPX4, a selenium-dependent protein, functions as a phospholipid hydroperoxidase, reducing phospholipid hydroperoxides (arachidonic acid/adrenic acid-PE-OOH) to their corresponding phospholipids [12, 31]. The primary role of GPX4 is to utilize the tripeptide GSH as a cofactor to detoxify lipid hydroperoxides formed during oxidative stress, thereby preserving membrane integrity.

A significant number of ferroptosis-inducing agents are regulated by the NRF2 pathway, which is maintained at low levels by three E3 ubiquitin ligases: Kelch-like ECH-associated protein 1-CUL3-RBX1, $SCF^{\beta TrCP}$, and synoviolin/Hrd1. Under normal conditions, the protein level of the nuclear transcription factor NFE2L2 is maintained at a low level due to its binding with Kelch-like ECH-associated protein 1, leading to its ubiquitination and degradation [32]. As an E3 ubiquitin ligase, HECT and RCC1-like domain containing protein 2 (HERC2) plays a critical role in iron homeostasis by targeting nuclear receptor coactivator 4 (NCOA4), a ferritin receptor that mediates ferritin autophagy, and F-box and leucine-rich repeat protein 5 (FBXL5),a regulator of iron regulatory proteins IRP1/2 that mediates ferritin synthesis, for degradation, thereby downregulating iron levels. NRF2 upregulates HERC2 to counteract ferroptosis [33]. Moreover, NRF2 positively regulates xCT (SLC7A11, a subunit of system $Xc^-$), which facilitates the exchange of extracellular cystine for intracellular glutamate. NRF2 also transcriptionally regulates thioredoxin and thioredoxin reductase 1, both of which facilitate the reduction of cystine to cysteine. Furthermore, NRF2 enhances the expression of solute carrier family A1 member 5, a critical transporter for glutamine uptake, thereby contributing to the biosynthesis of GSH (**Figure 1**).

### Potential crosstalk between ferroptosis and other forms of cell death in sepsis

A complex interplay exists between ferroptosis and other forms of cell death, including apoptosis, necrosis, and pyroptosis, during sepsis, influencing both disease pathogenesis and therapeutic strategies.

• Ferroptosis and Apoptosis: Apoptosis, a programmed cell death modality orchestrated by caspase family proteases, involves key processes such as cytochrome C release, caspase-3 and caspase-7 activation, nuclear condensation, and membrane blebbing [34]. In sepsis, ferroptosis and apoptosis may reciprocally influence each other. Lipid peroxidation products generated during ferroptosis, such as MDA and ROS can activate apoptotic signaling pathways, including the mitochondrial pathway [35]. For instance, in sepsis-associated myocardial injury, upregulation of ferroptosis markers, such as cyclooxygenase-2, is closely associated with increased cardiomyocyte apoptosis. Conversely, the loss of cell membrane integrity during apop-

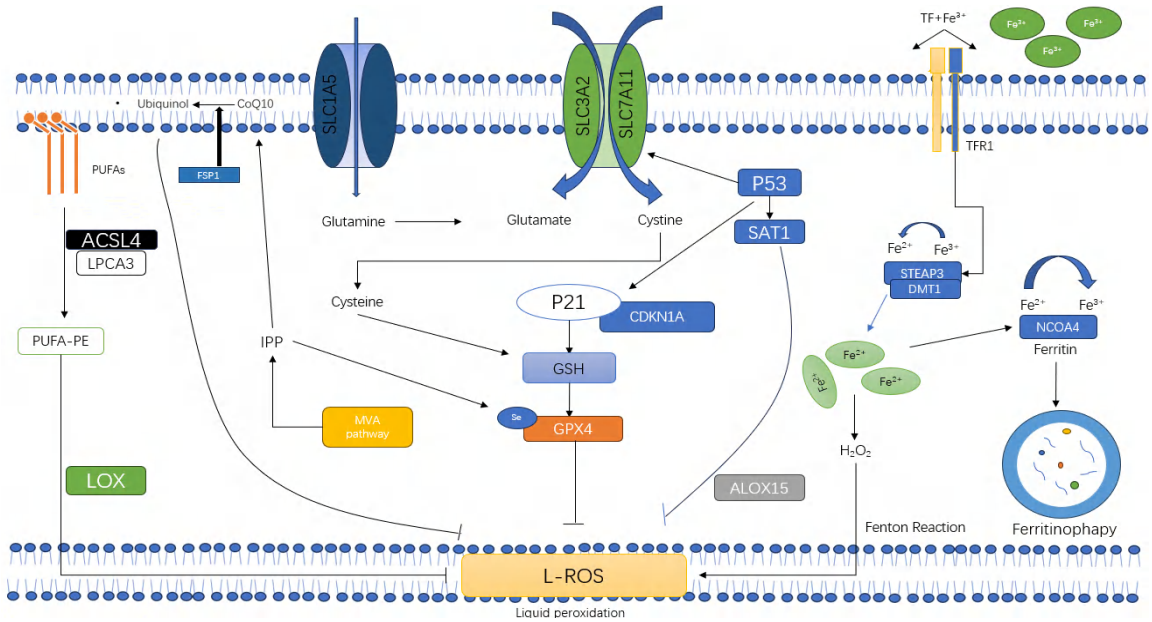


**Figure 1. Ferroptosis-related signaling pathways.** GSH, glutathione; GPX4, glutathione peroxidase 4; SLC7A11, solute carrier family 7 member 11; SLC3A2, solute carrier family 3 member 2; SLC1A5, solute carrier family 1 member 5; TF, transferrin; TFR1, the transferrin receptor 1; NCOA4, nuclear receptor coactivator 4; DMT1, divalent metal transporter 1; P53, a tumor suppressor protein; SAT1, spermine $N^1$-acetyltransferase 1; CDKN1A, Cyclin-dependent kinase inhibitor 1A; ALOX15, 15-lipoxygenase; PUFA-PE, oxidation of polyunsaturated phosphatidylethanolamines; LOX, lectin-like oxidized low-density lipoprotein receptor; ACSL4, acyl-CoA, synthetase long chain family member 4; PUFAs, polyunsaturated fatty acids.

tosis may also promote the release of iron ions, thereby exacerbating ferroptosis [36].

• Ferroptosis and Necrosis: Ferroptosis and necrosis may also interact in sepsis. Necrosis, a more drastic form of cell death than ferroptosis, is typically characterized by the loss of cell membrane integrity, cellular swelling, and leakage of cellular contents [21, 37]. In ferroptosis, the intracellular accumulation of iron ions promotes lipid peroxidation, leading to damage and dysfunction of the cell membrane. Necrosis, however, is more often triggered by severe changes in the cellular environment (e.g., ischemia, hypoxia, infection), resulting in the disruption of cellular structures [38]. Concurrently, lipid peroxidation products (e.g., MDA) and ROS generated during ferroptosis can further damage the cell membrane, leading to the loss of membrane integrity and necrosis. The release of cellular contents during necrosis can exacerbate local inflammatory responses, which in turn may promote the occurrence of ferroptosis.

• Ferroptosis and Pyroptosis: Pyroptosis is a form of programmed cell death triggered by inflammasome activation. The central mechanisms involve the activation of caspase-1, cleavage of Gasdermin D, and the formation of membrane pores, resulting in the release of cellular contents and inflammatory responses [39]. In the context of ferroptosis, the generation of ROS and lipid peroxidation products exacerbates intracellular oxidative stress [40]. This increased stress may activate inflammasomes, subsequently triggering pyroptosis. Concurrently, the release of cellular contents during pyroptosis further amplifies the localized inflammatory response, which could, in turn, promote ferroptosis through various pathways [41].

## Ferroptosis in sepsis

Sepsis is a life-threatening condition triggered by an uncontrolled immune response, leading to organ dysfunction and often causing mortality in critically ill patients, particularly in Intensive Care Units [42]. It is defined as a clinical syndrome characterized by a systemic inflammatory response to infection. The immune response in sepsis is multifactorial, typically described as comprising two components [43]. The first component is a state of excessive inflammation, driven by the systemic inflammatory response to infection [44]. Damage-associated molecular patterns interact with pattern recognition receptors expressed on the surface of immune cells, resulting in the release of pro-inflammatory cytokines and chemokines, as well as the mobilization of immune cells to the site of inflammation, thereby exacerbating inflammation and organ dysfunction. The second component is an immunosuppressive phase,

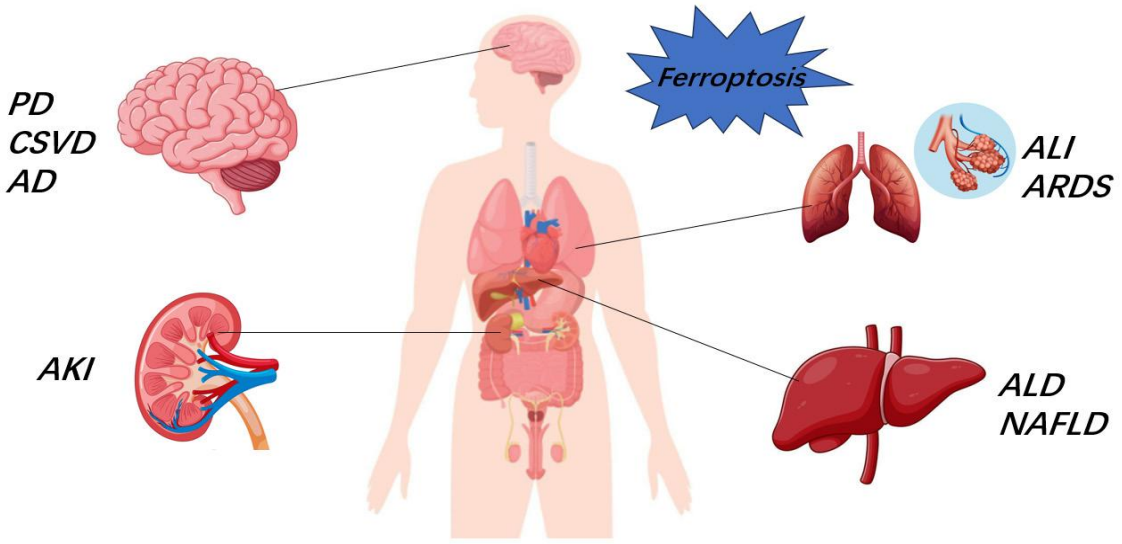


**Figure 2. Diseases associated with ferroptosis.** PD, Parkinson's disease; CSVD, cerebral small vessel disease; AD, Alzheimer's disease; ALI, acute lung injury; ARDS, acute respiratory distress syndrome; AKI, acute kidney injury; ALD, alcoholic liver disease; NAFLD, nonalcoholic fatty liver disease.

which increases susceptibility to secondary infections [45]. Various forms of cell death play a critical role in both the excessive inflammation and the immunosuppressive state in sepsis, regardless of whether they involve immune or non-immune cells. Ferroptosis alters the immune response in both immune and non-immune cells and significantly contributes to disease progression.

### *Iron overload and its role in bacterial infection-induced sepsis*

Iron plays a crucial role in the metabolic processes of pathogenic microorganisms during sepsis. Certain bacteria, such as Escherichia coli and Klebsiella pneumoniae, are highly dependent on iron for virulence [46-48]. In the early stages of infection, iron-induced cell death in intestinal epithelial cells compromises the intestinal barrier, facilitating the entry of harmful gut bacteria and toxins into the bloodstream and extravascular tissues [49]. To mitigate iron uptake and alleviate infection, the body employs a defensive strategy that involves reducing serum iron levels, increasing tissue iron sequestration, and sequestering iron from bacteria through cytoplasmic mechanisms [50]. However, excessive intracellular free iron not only exacerbates the inflammatory response but also induces cell death through oxidative reactions. During sepsis, macrophages, essential immune cells, are activated via Toll-like receptors, thereby activating the immune response. Iron-induced cell death can impair macrophage autophagy via the PI3K/AKT/mTOR signaling pathway, disrupting the clearance of inflammatory mediators and promoting the release of damage-associated molecular patterns, ultimately intensifying the inflammatory response [51]. Lipid peroxidation in sepsis arises during the immune response to infection, leading to elevated levels of ROS and reactive oxygen intermediates, which interact with lipids in cell membranes and trigger lipid peroxidation. The byproducts of this process, such as MDA and 4-hydroxy-2-nonenal (HNE), further exacerbate sepsis [52]. Notably, HNE can inhibit ATP-triggered cell death by suppressing GPX4, while promoting ferroptosis-related responses [53]. In macrophages, ferroptosis is characterized by substantial increases in both intracellular iron and lipid peroxidation. Ferroptosis inducers, such as RSL3, sulfasalazine, and acetaminophen, enhance the bactericidal activity of macrophages [54]. However, the inhibition of GPX4 caused by lipid peroxidation can disrupt the binding of T cell receptor (TCR) to T cells, affecting the homeostatic survival of CD8 T cells and the proliferation of $CD4^+$ and $CD8^+$ T cells in response to infection, thereby diminishing the body's immune response to infections. Thus, ferroptosis acts as a double-edged sword: while it facilitates bacterial entry into the body and contributes to the initiation of sepsis, it can also help immune cells eliminate pathogens [55].

### *Ferroptosis in organ damage during sepsis*

Recent studies have highlighted the significant role of ferroptosis in the pathogenesis of sepsis [56, 57]. Given that sepsis affects multiple organ systems, ferroptosis contributes notably to organ damage across various sites (**Figure 2**) [58].

*The role of ferroptosis in sepsis-related brain injury*

Ferroptosis is crucial in the context of sepsis-related brain injury. The systemic inflammatory response triggered by sepsis can alter the brain's microenvironment, disrupting the iron metabolism balance in brain cells. During septic encephalopathy, the abnormal accumulation of iron ions can generate substantial amounts of hydroxyl radicals through the Fenton reaction, resulting in lipid peroxidation, membrane damage, and ultimately neuronal injury and cognitive dysfunction [59]. Studies have shown that the levels of iron ions in the brain tissue of sepsis patients are significantly elevated and positively correlate with the severity of neurological injury [60, 61]. As a catalyst in the Fenton reaction, the excessive accumulation of iron ions can exacerbate oxidative stress, further promoting neuronal damage. One key factor in sepsis-related brain injury is the disruption of the blood-brain barrier [2]. The abnormal translocation of iron ions across the blood-brain barrier intensifies oxidative stress in the brain, promoting ferroptosis and exacerbating brain tissue damage.

*The role of ferroptosis in sepsis-related lung injury*

Acute lung injury and acute respiratory distress syndrome are common complications in sepsis, with ferroptosis playing a significant role in their progression [62]. The accumulation of iron ions in alveolar epithelial cells and endothelial cells promotes lipid peroxidation, leading to cell membrane rupture and the disruption of the alveolar-capillary barrier. This causes pulmonary edema and impaired gas exchange. Excessive accumulation of iron ions in these cells increases the permeability of the alveolar-capillary barrier by activating lipid peroxidation pathways, representing a critical mechanism in the development of acute lung injury/acute respiratory distress syndrome. Additionally, iron ions can influence the progression of sepsis-related lung injury by modulating pulmonary inflammatory responses [63]. Iron-induced oxidative stress activates the release of inflammatory mediators such as tumor necrosis factor-alpha and interleukin-6, exacerbating lung inflammation.

*The role of ferroptosis in sepsis-related liver injury*

The liver, as the primary organ involved in iron metabolism, is highly susceptible to damage during sepsis. Ferroptosis significantly impacts liver function, primarily through direct damage to hepatocytes. Extensive hepatocyte death disrupts the liver's metabolic, detoxification, and synthetic functions, further exacerbating the pathological progression of sepsis. Additionally, the inflammatory response triggered by ferroptosis recruits immune cells, leading to disturbances in the hepatic microenvironment and impairing liver function [64, 65]. The accumulation of iron ions within hepatocytes promotes oxidative stress and lipid peroxidation, resulting in hepatocyte injury and liver dysfunction [66]. Furthermore, iron ions modulate the liver's inflammatory response, with iron-induced oxidative stress enhancing the release of inflammatory cytokines, intensifying liver inflammation and damage.

*The role of ferroptosis in sepsis-related kidney injury*

The kidneys are another organ vulnerable to damage during sepsis. Renal tubular epithelial cells are particularly sensitive to iron overload in the septic state [67]. During the septic state, the body's inflammatory response leads to an increased release of iron ions, especially within the renal tubular cells. The accumulation of iron ions in these cells promotes lipid peroxidation, resulting in cellular dysfunction and tubular necrosis, which impairs the kidneys' filtration and excretion capabilities. Iron overload within renal tubular epithelial cells contributes to cellular damage and dysfunction through the activation of oxidative stress pathways, representing a critical mechanism underlying sepsis-associated acute kidney injury [67]. Additionally, iron ions can influence the progression of sepsis-related kidney injury by modulating the renal inflammatory response. Iron-induced oxidative stress triggers the release of inflammatory mediators, exacerbating renal inflammation and damage.

## Targeting ferroptosis for sepsis treatment

### *Targeted therapies for ferroptosis*

Targeting ferroptosis in sepsis involves modulating key mechanisms such as iron ion accumulation, lipid peroxidation, and the inhibition of GPX4 activity [68]. Potential therapeutic targets include:

• ACSL4 and lysolipid phosphatidylcholine acyltransferases 3: these enzymes are important drivers of ferroptosis. They promote lipid peroxidation by catalyzing the esterification and oxidation of polyunsaturated fatty acids. Studies have found that inhibiting the activity of ACSL4

can reduce tissue damage associated with ferroptosis [69-71].

• SLC7A11: As a key subunit of the system $Xc^-$, SLC7A11 affects intracellular glutathione levels by regulating the uptake of cystine, thereby regulating the sensitivity of ferroptosis.

• Ferroptosis suppressor protein 1: Ferroptosis suppressor protein 1 protects cells from ferroptosis by regulating ubiquinone levels) [38, 72, 73]. Targeting ferroptosis suppressor protein 1 has been shown to enhance the sensitivity of lung cancer cells to radiation.

Ferroptosis therapies in clinical settings include ferroptosis agonists and inhibitors (e.g., Fer-1). Ferroptosis agonists induce cell ferroptosis by promoting iron ion accumulation, lipid peroxidation, or inhibiting antioxidant systems. For example, arginine has been identified as a ferroptosis promoter, which regulates ferroptosis through polyamine metabolism, making cancer cells more susceptible to radiotherapy and chemotherapy [74]. Ferroptosis inhibitors protect cells from damage by blocking key ferroptotic pathways. For example, preserving GPX4 or regulating iron metabolism-related proteins (e.g., SLC7A11) can reduce intracellular lipid peroxidation and iron ion accumulation. In sepsis research, the ferroptosis inhibitor Fer-1 has been shown to significantly restore pericyte viability, reduce lipid ROS content, lower inflammatory cytokine levels, and ameliorate pulmonary vascular barrier dysfunction [75].

***Other factors influencing ferroptosis susceptibility and therapeutic response***

• Genetic factors: Studies have revealed that genetic variations play a significant role in determining cellular sensitivity to ferroptosis [76-78]. For instance, p53 tumor suppressor can sensitize cells to ferroptosis by inhibiting the transcription of the *SLC7A11* gene [79]. Conversely, NRF2 can protect cells from ferroptosis by upregulating *SLC7A11*.

• Polyamine metabolism: Studies from Fudan University have discovered that iron overload activates ornithine decarboxylase 1 through the WNT/MYC signaling pathway, promoting polyamine synthesis, which forms a positive feedback loop that amplifies ferroptosis. Interestingly, cancer cells with polyamine overload are not only more susceptible to ferroptosis but can also transmit this sensitivity to surrounding cells via extracellular vesicles [74].

***Comorbidities influencing ferroptosis susceptibility***

• Depression: Increased iron deposition in the brain of patients with depression has been linked to increased susceptibility to ferroptosis. Iron catalyzes the generation of harmful hydroxyl radicals through the Fenton reaction, leading to DNA oxidation and lipid damage, which in turn compromise neurotransmitter synthesis and the function of nerve cells [80].

• Diabetes: In type 1 diabetes, pancreatic β-cells are extremely sensitive to oxidative stress. Stimuli such as hyperglycemia and hyperlipidemia significantly increase oxidative stress in β-cells, thereby amplifying their susceptibility to ferroptosis [81]. In patients with hereditary hemochromatosis, the accumulation of iron in β-cells can induce cell death, leading to the development of diabetes.

• Cardiovascular Diseases: Cardiovascular diseases are closely associated with ferroptosis, oxidative stress, and inflammatory responses. Research indicates that ferroptosis may exacerbate the pathological processes of cardiovascular diseases by augmenting oxidative stress and inflammatory responses [82, 83].

• Tumors: In the tumor microenvironment, dysregulation of iron metabolism and increased oxidative stress in tumor cells render them more susceptible to ferroptosis. Studies have shown that targeting ferroptosis pathways can enhance the sensitivity of tumor cells to chemotherapy and radiotherapy [84, 85].

The response to ferroptosis inhibitors or inducers varies among individuals. For instance, neonatal hypoxic-ischemic encephalopathy can be mitigated by Fer-1, but this therapeutic effect is influenced by genetic factors and the severity of the disease. Combining ferroptosis inhibitors with other therapeutic modalities, such as chemotherapy or radiotherapy, has shown promise in improving therapeutic outcomes. For example, in the treatment of gliomas, combined use of ferroptosis inhibitors and radiotherapy can enhance therapeutic efficacy [86].

## Controversies and unresolved issues in the study of ferroptosis in sepsis

While many studies suggest ferroptosis plays a significant role in the development and progression of sepsis, there remains a debate regarding whether ferroptosis is a primary cause of sepsis or merely a secondary event in the disease's pathophysiology. For instance, some studies have found that iron chelators can re-

duce iron ion concentrations, inhibit the occurrence of cellular ferroptosis, and thus improve the survival rate of sepsis patients [87, 88]. However, other studies propose that ferroptosis may be a consequence of systemic inflammatory response syndrome and multiple organ dysfunction syndrome, rather than a direct initiator of sepsis pathology [89, 90].

Currently, the detection methods for ferroptosis are not fully standardized, and there are differences in the detection indicators and methods used across studies. For example, some studies assess the occurrence of ferroptosis by detecting intracellular iron content and lipid peroxidation products (e.g., MDA levels), while other studies rely on specific cellular morphological changes, such as loss of cell membrane integrity or mitochondrial shrinkage [62]. This lack of uniformity complicates the interpretation of ferroptosis' role in sepsis and hinders the development of reliable biomarkers for clinical application [59].

The precise molecular mechanisms underlying ferroptosis, particularly in the context of sepsis, remain incompletely elucidated. While iron metabolism and lipid peroxidation are well-established triggers, the roles of key regulatory proteins, such as ACSL4 and GPX4, as well as the intricate signaling pathways involved (e.g., Nrf2/ARE pathway, ferroptosis-related autophagy), require further investigation [74].

Sepsis can induce multi-organ dysfunction, yet the roles and mechanisms of ferroptosis in different organs remain unclear. For example, whether ferroptosis plays a consistent role in sepsis-associated complications like myocardial injury and acute kidney injury, as well as the specific mechanisms and targets in different organs, warrants further study.

Although some studies have explored the potential therapeutic value of ferroptosis inhibitors in sepsis treatment, effective clinical strategies and drugs are currently lacking [91, 92]. For example, while iron chelators have demonstrated therapeutic potential in experimental studies, their clinical application faces challenges, including drug toxicity, administration routes, and dosage issues. Moreover, formulating personalized ferroptosis intervention strategies based on individual differences, such as genetic background and disease severity, is a critical issue that needs to be addressed.

## III Summary

This review summarizes the mechanisms of ferroptosis, its primary biological responses, and its role in the pathogenesis of sepsis. Ferroptosis plays a significant role in the inflammatory processes of sepsis, contributing not only to direct cell damage but also to the amplification and persistence of the inflammatory response. However, the molecular mechanisms and regulatory networks governing ferroptosis in sepsis remain insufficiently understood. Further research is essential to unravel these processes, which could lead to novel therapeutic approaches for more effective clinical treatment. Additionally, the identification and validation of biomarkers related to ferroptosis hold promise for early diagnosis and precision treatment of sepsis. As our understanding of the mechanisms of ferroptosis continues to evolve, there is potential for it to bring new hope and direction in the clinical treatment of sepsis.

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## References

[1] Wang M, Gao Q, Guo S. Diagnostic and prognostic significance of apelin-13, APJ for sepsis in the emergency department: A prospective study. Heliyon 2024;10(7):e28620.

[2] Pan S, Lv Z, Wang R, et al. Sepsis-Induced Brain Dysfunction: Pathogenesis, Diagnosis, and Treatment. Oxid Med Cell Longev 2022;2022:1328729.

[3] Rudd KE, Johnson SC, Agesa KM, et al. Global, regional, and national sepsis incidence and mortality, 1990-2017: analysis for the Global Burden of Disease Study. Lancet 2020;395(10219):200-211.

[4] Gańczak M, Miazgowski T, Kożybska M, et al. Changes in disease burden in Poland between 1990-2017 in comparison with other Central European countries: A systematic analysis for the Global Burden of Disease Study 2017. PLoS One 2020;15(3):e0226766.

[5] Xu J, Tao L, Jiang L, et al. Moderate Hypothermia Alleviates Sepsis-Associated Acute Lung Injury by Suppressing Ferroptosis Induced by Excessive Inflammation and Oxidative Stress via the Keap1/GSK3β/Nrf2/GPX4 Signaling Pathway. J Inflamm Res 2024;17(0):7687-7704.

[6] Evans L, Rhodes A, Alhazzani W, et al.

Surviving sepsis campaign: international guidelines for management of sepsis and septic shock 2021. Intensive Care Med 2021;47(11):1181-1247.
[7] Oczkowski S, Alshamsi F, Belley-Cote E, et al. Surviving Sepsis Campaign Guidelines 2021: highlights for the practicing clinician. Pol Arch Intern Med 2022;132(7-8):16290.
[8] Vandewalle J, Libert C. Sepsis: a failing starvation response. Trends Endocrinol Metab 2022;33(4):292-304.
[9] Stockwell BR. Ferroptosis turns 10: Emerging mechanisms, physiological functions, and therapeutic applications. Cell 2022;185(14):2401-2421.
[10] Chen T, Liang L, Wang Y, et al. Ferroptosis and cuproptposis in kidney Diseases: dysfunction of cell metabolism. Apoptosis: an international journal on programmed cell death 2024;29(3-4):289-302.
[11] Gan B. ACSL4, PUFA, and ferroptosis: new arsenal in anti-tumor immunity. Signal transduction and targeted therapy 2022;7(1):128.
[12] Gao M, Yi J, Zhu J, et al. Role of Mitochondria in Ferroptosis. Mol Cell 2019;73(2):354-363. e353.
[13] Liu J, Kang R, Tang D. Signaling pathways and defense mechanisms of ferroptosis. Febs j 2022;289(22):7038-7050.
[14] Wang L, Liu Y, Du T, et al. ATF3 promotes erastin-induced ferroptosis by suppressing system Xc(). Cell Death Differ 2020;27(2):662-675.
[15] Liang D, Feng Y, Zandkarimi F, et al. Ferroptosis surveillance independent of GPX4 and differentially regulated by sex hormones. Cell 2023;186(13):2748-2764.e2722.
[16] Stockwell BR, Friedmann Angeli JP, Bayir H, et al. Ferroptosis: A Regulated Cell Death Nexus Linking Metabolism, Redox Biology, and Disease. Cell 2017;171(2):273-285.
[17] Sun Y, Chen P, Zhai B, et al. The emerging role of ferroptosis in inflammation. Biomed Pharmacother 2020;127:110108.
[18] Liang D, Minikes AM, Jiang X. Ferroptosis at the intersection of lipid metabolism and cellular signaling. Mol Cell 2022;82(12):2215-2227.
[19] Battaglia AM, Chirillo R, Aversa I, et al. Ferroptosis and Cancer: Mitochondria Meet the "Iron Maiden" Cell Death. Cells 2020;9(6):1505.
[20] Mortensen MS, Ruiz J, Watts JL. Polyunsaturated Fatty Acids Drive Lipid Peroxidation during Ferroptosis. Cells 2023;12(5):804.
[21] Li J, Cao F, Yin HL, et al. Ferroptosis: past, present and future. Cell Death Dis 2020;11(2):88.
[22] Tang D, Chen X, Kang R, et al. Ferroptosis: molecular mechanisms and health implications. Cell Res 2021;31(2):107-125.
[23] Bogdan AR, Miyazawa M, Hashimoto K, et al. Regulators of Iron Homeostasis: New Players in Metabolism, Cell Death, and Disease. Trends Biochem Sci 2016;41(3):274-286.
[24] Outten FW, Theil EC. Iron-based redox switches in biology. Antioxid Redox Signal 2009;11(5):1029-1046.
[25] Kerins MJ, Ooi A. The Roles of NRF2 in Modulating Cellular Iron Homeostasis. Antioxid Redox Signal 2018;29(17):1756-1773.
[26] Jiang X, Stockwell BR, Conrad M. Ferroptosis: mechanisms, biology and role in disease. Nat Rev Mol Cell Biol 2021;22(4):266-282.
[27] Dixon SJ, Lemberg KM, Lamprecht MR, et al. Ferroptosis: an iron-dependent form of nonapoptotic cell death. Cell 2012;149(5):1060-1072.
[28] Kennedy L, Sandhu JK, Harper ME, et al. Role of Glutathione in Cancer: From Mechanisms to Therapies. Biomolecules 2020;10(10):1429.
[29] Yao J, Li J, Xiong D, et al. Development of a highly efficient and specific L-theanine synthase. Appl Microbiol Biotechnol 2020;104(8):3417-3431.
[30] He F, Zhang P, Liu J, et al. ATF4 suppresses hepatocarcinogenesis by inducing SLC7A11 (xCT) to block stress-related ferroptosis. J Hepatol 2023;79(2):362-377.
[31] Ursini F, Maiorino M. Lipid peroxidation and ferroptosis: The role of GSH and GPx4. Free Radic Biol Med 2020;152:175-185.
[32] Rojo de la Vega M, Chapman E, Zhang DD. NRF2 and the Hallmarks of Cancer. Cancer Cell 2018;34(1):21-43.
[33] Anandhan A, Dodson M, Shakya A, et al. NRF2 controls iron homeostasis and ferroptosis through HERC2 and VAMP8. Sci Adv 2023;9(5):eade9585.
[34] Ai Y, Meng Y, Yan B, et al. The biochemical pathways of apoptotic, necroptotic, pyroptotic, and ferroptotic cell death. Mol Cell 2024;84(1):170-179.
[35] Li J, Jia YC, Ding YX, et al. The crosstalk between ferroptosis and mitochondrial dynamic regulatory networks. Int J Biol Sci 2023;19(9):2756-2771.
[36] Dai E, Chen X, Linkermann A, et al. A guideline on the molecular ecosystem regulating ferroptosis. Nat Cell Biol 2024;26(9):1447-1457.
[37] Hassannia B, Van Coillie S, Vanden Berghe T. Ferroptosis: Biological Rust of Lipid Membranes. Antioxid Redox Signal 2021;35(6):487-509.
[38] Feng H, Yu J, Xu Z, et al. SLC7A9 suppression increases chemosensitivity by inducing

ferroptosis via the inhibition of cystine transport in gastric cancer. EBioMedicine 2024;109:105375.
[39] Tan Y, Chen Q, Li X, et al. Pyroptosis: a new paradigm of cell death for fighting against cancer. J Exp Clin Cancer Res 2021;40(1):153.
[40] Jin X, Tang J, Qiu X, et al. Ferroptosis: Emerging mechanisms, biological function, and therapeutic potential in cancer and inflammation. Cell Death Discov 2024;10(1):45.
[41] Newton K, Strasser A, Kayagaki N, et al. Cell death. Cell 2024;187(2):235-256.
[42] Srzić I, Nesek Adam V, Tunjić Pejak D. SEPSIS DEFINITION: WHAT'S NEW IN THE TREATMENT GUIDELINES. Acta Clin Croat 2022;61(Suppl 1):67-72.
[43] Shimizu J, Murao A, Nofi C, et al. Extracellular CIRP Promotes GPX4-Mediated Ferroptosis in Sepsis. Front Immunol 2022;13:903859.
[44] Aziz M, Jacob A, Yang WL, et al. Current trends in inflammatory and immunomodulatory mediators in sepsis. J Leukoc Biol 2013;93(3):329-342.
[45] Hotchkiss RS, Monneret G, Payen D. Immunosuppression in sepsis: a novel understanding of the disorder and a new therapeutic approach. Lancet Infect Dis 2013;13(3):260-268.
[46] Snyder CC, Barton JR, Habli M, et al. Severe sepsis and septic shock in pregnancy: indications for delivery and maternal and perinatal outcomes. J Matern Fetal Neonatal Med 2013;26(5):503-506.
[47] Barton JR, Sibai BM. Severe sepsis and septic shock in pregnancy. Obstet Gynecol 2012;120(3):689-706.
[48] Postelnicu R, Evans L. Monitoring of the physical exam in sepsis. Curr Opin Crit Care 2017;23(3):232-236.
[49] Yang T, Feng X, Zhao Y, et al. Dexmedetomidine Enhances Autophagy via α2-AR/AMPK/mTOR Pathway to Inhibit the Activation of NLRP3 Inflammasome and Subsequently Alleviates Lipopolysaccharide-Induced Acute Kidney Injury. Front Pharmacol 2020;11:790.
[50] Ma D, Jiang P, Jiang Y, et al. Effects of Lipid Peroxidation-Mediated Ferroptosis on Severe Acute Pancreatitis-Induced Intestinal Barrier Injury and Bacterial Translocation. Oxid Med Cell Longev 2021;2021:6644576.
[51] Wang J, Zhu Q, Wang Y, et al. Irisin protects against sepsis-associated encephalopathy by suppressing ferroptosis via activation of the Nrf2/GPX4 signal axis. Free Radic Biol Med 2022;187:171-184.
[52] Huang Q, Ding Y, Fang C, et al. The Emerging Role of Ferroptosis in Sepsis, Opportunity or Challenge? Infect Drug Resist 2023;16:5551-5562.
[53] Li F, Wang S, Zhou Y, et al. [Signal transducer and activator of transcription 6 mediates skeletal muscle cell injury in septic mice by regulating ferroptosis]. Zhonghua Wei Zhong Bing Ji Jiu Yi Xue 2023;35(8):813-817.
[54] Ma R, Fang L, Chen L, et al. Ferroptotic stress promotes macrophages against intracellular bacteria. Theranostics 2022;12(5):2266-2289.
[55] Matsushita M, Freigang S, Schneider C, et al. T cell lipid peroxidation induces ferroptosis and prevents immunity to infection. J Exp Med 2015;212(4):555-568.
[56] Lai K, Song C, Gao M, et al. Uridine Alleviates Sepsis-Induced Acute Lung Injury by Inhibiting Ferroptosis of Macrophage. Int J Mol Sci 2023;24(6):5093.
[57] Zhang H, Liu J, Zhou Y, et al. Neutrophil extracellular traps mediate m(6)A modification and regulates sepsis-associated acute lung injury by activating ferroptosis in alveolar epithelial cells. Int J Biol Sci 2022;18(8):3337-3357.
[58] Netea MG, Giamarellos-Bourboulis EJ, Domínguez-Andrés J, et al. Trained Immunity: a Tool for Reducing Susceptibility to and the Severity of SARS-CoV-2 Infection. Cell 2020;181(5):969-977.
[59] Zeng F, Nijiati S, Tang L, et al. Ferroptosis Detection: From Approaches to Applications. Angew Chem Int Ed Engl 2023;62(35):e202300379.
[60] Du L, Wu Y, Fan Z, et al. The Role of Ferroptosis in Nervous System Disorders. J Integr Neurosci 2023;22(1):19.
[61] David S, Jhelum P, Ryan F, et al. Dysregulation of Iron Homeostasis in the Central Nervous System and the Role of Ferroptosis in Neurodegenerative Disorders. Antioxid Redox Signal 2022;37(1-3):150-170.
[62] Wu D, Spencer CB, Ortoga L, et al. Histone lactylation-regulated METTL3 promotes ferroptosis via m6A-modification on ACSL4 in sepsis-associated lung injury. Redox Biol 2024;74:103194.
[63] Xu F, Xie J, Mou W, et al. The VDR/FFAR2 axis mitigates sepsis-induced lung injury by suppressing macrophage lipid peroxidation. Int Immunopharmacol 2024;143(Pt 2):113328.
[64] Valenti L, Corradini E, Adams LA, et al. Consensus Statement on the definition and classification of metabolic hyperferritinaemia. Nat Rev Endocrinol 2023;19(5):299-310.
[65] Valenti L, Corradini E, Adams LA, et al. Author Correction: Consensus Statement on the definition and classification of metabolic hyperferritinaemia. Nat Rev Endocrinol

2024;20(3):185.
[66] Zhu Z, Chambers S, Bhatia M. Suppressing the Substance P-NK1R Signalling Protects Mice against Sepsis-Associated Acute Inflammatory Injury and Ferroptosis in the Liver and Lungs. Antioxidants (Basel) 2024;13(3):300.
[67] Vlahakos D, Arkadopoulos N, Kostopanagiotou G, et al. Deferoxamine attenuates lipid peroxidation, blocks interleukin-6 production, ameliorates sepsis inflammatory response syndrome, and confers renoprotection after acute hepatic ischemia in pigs. Artif Organs 2012;36(4):400-408.
[68] Meng Y, Sun H, Li Y, et al. Targeting Ferroptosis by Ubiquitin System Enzymes: A Potential Therapeutic Strategy in Cancer. Int J Biol Sci 2022;18(14):5475-5488.
[69] Cui Y, Zhang Y, Zhao X, et al. ACSL4 exacerbates ischemic stroke by promoting ferroptosis-induced brain injury and neuroinflammation. Brain Behav Immun 2021;93:312-321.
[70] Wang Y, Zhang M, Bi R, et al. ACSL4 deficiency confers protection against ferroptosis-mediated acute kidney injury. Redox Biol 2022;51:102262.
[71] Liao P, Wang W, Wang W, et al. CD8(+) T cells and fatty acids orchestrate tumor ferroptosis and immunity via ACSL4. Cancer Cell 2022;40(4):365-378.e366.
[72] Koppula P, Lei G, Zhang Y, et al. A targetable CoQ-FSP1 axis drives ferroptosis- and radiation-resistance in KEAP1 inactive lung cancers. Nat Commun 2022;13(1):2206.
[73] Bersuker K, Hendricks JM, Li Z, et al. The CoQ oxidoreductase FSP1 acts parallel to GPX4 to inhibit ferroptosis. Nature 2019;575(7784):688-692.
[74] Bi G, Liang J, Bian Y, et al. Polyamine-mediated ferroptosis amplification acts as a targetable vulnerability in cancer. Nat Commun 2024;15(1):2461.
[75] Liu Y, Bao D, She H, et al. Role of Hippo/ACSL4 axis in ferroptosis-induced pericyte loss and vascular dysfunction in sepsis. Redox Biol 2024;78:103353.
[76] Huang B, Wang H, Liu S, et al. Palmitoylation-dependent regulation of GPX4 suppresses ferroptosis. Nat Commun 2025;16(1):867.
[77] Wang G, Qin S, Chen L, et al. Butyrate dictates ferroptosis sensitivity through FFAR2-mTOR signaling. Cell Death Dis 2023;14(4):292.
[78] Dixon SJ, Olzmann JA. The cell biology of ferroptosis. Nat Rev Mol Cell Biol 2024;25(6):424-442.
[79] Jiang L, Kon N, Li T, et al. Ferroptosis as a p53-mediated activity during tumour suppression. Nature 2015;520(7545):57-62.
[80] Zhang Y, Wang M, Chang W. Iron dyshomeostasis and ferroptosis in Alzheimer's disease: Molecular mechanisms of cell death and novel therapeutic drugs and targets for AD. Front Pharmacol 2022;13:983623.
[81] Dai L, Wang Q. Targeting ferroptosis: opportunities and challenges of mesenchymal stem cell therapy for type 1 diabetes mellitus. Stem Cell Res Ther 2025;16(1):47.
[82] Li N, Jiang W, Wang W, et al. Ferroptosis and its emerging roles in cardiovascular diseases. Pharmacol Res 2021;166:105466.
[83] Fang X, Ardehali H, Min J, et al. The molecular and metabolic landscape of iron and ferroptosis in cardiovascular disease. Nat Rev Cardiol 2023;20(1):7-23.
[84] Wang Y, Wang W, Zhang Y, et al. Targeting ferroptosis offers therapy choice in sepsis-associated acute lung injury. Eur J Med Chem 2025;283:117152.
[85] Sun S, Shen J, Jiang J, et al. Targeting ferroptosis opens new avenues for the development of novel therapeutics. Signal Transduct Target Ther 2023;8(1):372.
[86] Wan S, Zhang G, Liu R, et al. Pyroptosis, ferroptosis, and autophagy cross-talk in glioblastoma opens up new avenues for glioblastoma treatment. Cell Commun Signal 2023;21(1):115.
[87] Tao L, Yang X, Ge C, et al. Integrative clinical and preclinical studies identify FerroTerminator1 as a potent therapeutic drug for MASH. Cell Metab 2024;36(10):2190-2206.e2195.
[88] Chen Y, Li X, Wang S, et al. Targeting Iron Metabolism and Ferroptosis as Novel Therapeutic Approaches in Cardiovascular Diseases. Nutrients 2023;15(3):591.
[89] Li H, Wu D, Zhang H, et al. New insights into regulatory cell death and acute pancreatitis. Heliyon 2023;9(7):e18036.
[90] Van Coillie S, Van San E, Goetschalckx I, et al. Targeting ferroptosis protects against experimental (multi)organ dysfunction and death. Nat Commun 2022;13(1):1046.
[91] Xl L, Gy Z, R G, et al. Ferroptosis in sepsis: The mechanism, the role and the therapeutic potential. Front Immunol 2022;13:956361.
[92] Huo L, Liu C, Yuan Y, et al. Pharmacological inhibition of ferroptosis as a therapeutic target for sepsis-associated organ damage. Eur J Med Chem 2023;257:115438.