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ORIGINAL RESEARCH

# Multi-Omics Analysis Identifies Immune Regulatory Networks in Sepsis-Associated Liver Injury: Experimental Validation and Clinical Relevance

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**Purpose:** Sepsis-induced liver injury (SILI) significantly contributes to mortality, yet its underlying immune mechanisms remain poorly understood. This study aimed to identify key immune-related genes (IRGs) driving T cell-mediated responses in SILI and evaluate their diagnostic potential.

**Methods:** Cecal ligation and puncture (CLP) was performed to establish a murine sepsis model (n=7/group), with sham-operated controls. Serum IL-1β and lactate levels were quantified via ELISA. Public transcriptomic datasets (GSE26440, GSE26378, GSE25504, GSE28750; 180 sepsis vs 53 controls) were analyzed to identify differentially expressed IRGs (DEIRGs). Functional enrichment (GO/KEGG), protein-protein interaction (PPI) networks, and single-cell RNA sequencing (scRNA-seq) were integrated to prioritize T cell-associated genes. Flow cytometry assessed immune cell subsets (CD3+ T cells, CD19+ B cells, NK1.1+ NK cells, F4/80+ macrophages) in murine blood. Liver histopathology (HE staining) and cytokine expression (IL-1β/TNF-α; IHC) were evaluated.

**Results:** CLP mice exhibited elevated IL-1β ($P<0.01$) and lactate ($P<0.01$), confirming metabolic dysfunction and inflammation. Bioinformatics analysis identified 19 DEIRGs, with PPI networks implicating immune regulation (PPI enrichment $p=3.35\times10\text{-}6$). Flow cytometry confirmed T cell dominance (65.87% vs 63.85% in controls). scRNA-seq revealed four T cell-linked hub genes (FCER1G, IL2RB, PTGDR, XCL1). Protein-protein interaction (PPI) network analysis demonstrated that these genes form a synergistic regulatory network involving NF-κB, JAK-STAT, and other key pathways, with notable features including the IL2RB-XCL1 positive feedback loop and the opposing effects of PTGDR isoforms (DP1/DP2). Histopathology showed hepatic necrosis and inflammatory infiltration, correlating with upregulated IL-1β/TNF-α (IHC, $P<0.05$).

**Conclusion:** FCER1G, IL2RB, PTGDR, and XCL1 are novel T cell-related biomarkers of SILI, offering potential therapeutic targets. The study bridges bioinformatics predictions with experimental validation, advancing understanding of immune dysregulation in sepsis.

**Keywords:** sepsis, acute liver injury, immunity, T cells, RNA single cell sequencing

## Introduction

Sepsis represents a dysregulated host response to infection that leads to life-threatening organ dysfunction, constituting a major global health burden with persistently high morbidity and mortality rates.[1] Epidemiological data reveal that sepsis affects approximately 8.2% of pediatric intensive care unit admissions worldwide, with mortality reaching 25% across all age groups and economic regions.[2] The liver, as a central immunological organ, plays a dual role in sepsis pathogenesis - serving as both a primary defense against circulating pathogens and a vulnerable target for inflammatory injury.[3]

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During sepsis progression, pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) disseminate hematogenously to hepatic tissue through portal and systemic circulation.[4] This triggers complex immunopathological responses involving hepatic sinusoidal endothelial cells, Kupffer cells, and recruited immune cell populations including neutrophils, monocytes, and natural killer cells.[5] The liver attempts to maintain immunological homeostasis through counter-regulatory mechanisms involving IL-10, TGF-β, and regulatory T cells, but these compensatory responses often culminate in either uncontrolled inflammation or immunoparalysis.[6] Emerging evidence highlights the pivotal role of alarmins, particularly IL-33, in modulating this delicate balance through IL-33/LPO/8-OHdG/caspase-3 signaling pathways that influence both innate and adaptive immune responses.[7,8]

Despite significant advances in sepsis research, critical knowledge gaps persist regarding the precise molecular mechanisms governing immune dysregulation in sepsis-induced liver injury. The advent of high-throughput technologies, including single-cell RNA sequencing and systems biology approaches, has begun to unravel the complex immunological networks underlying this condition. However, comprehensive characterization of key immune regulatory genes and their functional interplay in septic hepatic dysfunction remains incomplete.

Here, we present an integrated investigation combining multi-omics analyses with experimental validation to systematically identify and characterize critical immune regulatory networks in sepsis-associated liver injury. Our study aims to: (i) delineate the molecular signatures of immune dysregulation in septic liver injury; (ii) identify novel biomarkers with diagnostic and prognostic potential; and (iii) elucidate potential therapeutic targets for immunomodulatory intervention. These findings may provide new insights into the pathophysiological mechanisms of sepsis-induced organ failure and inform the development of targeted treatment strategies.

## Materials and Methods

To investigate the inflammatory response induced by sepsis, we established a clinically relevant mouse model of sepsis-induced liver injury using cecal ligation and puncture (CLP), which effectively recapitulates the pathophysiological features of human sepsis. In this model, proinflammatory cytokines IL-1β and TNF-α served as key inflammatory markers, while accumulated lactate levels were measured to assess metabolic dysfunction. Bioinformatics approaches were employed to analyze metabolic pathways associated with sepsis-induced liver injury, with particular focus on the role of T cell-mediated immune regulation. Four sepsis-related datasets (GSE26440, GSE26378, GSE25504, and GSE28750) were retrieved from the Gene Expression Omnibus (GEO) database:Training set: GSE26440 (98 pediatric sepsis patients vs 32 controls) and GSE26378 (82 sepsis cases vs 21 controls), comprising whole blood gene expression profiles from 180 septic children and 53 healthy controls. Validation set: GSE25504 (28 sepsis patients vs 35 controls; sequencing platform GPL6947) and GSE28750 (21 sepsis cases vs 20 controls). Additionally, immune-related genes were obtained from the ImmPort database (https://www.immport.org/home). After removing duplicates, 19 sepsis-associated immune genes were identified. Single-cell sequencing analysis revealed that FCER1G, IL2RB, PTGDR, and XCL1 showed significant correlation with T cell function ($P<0.05$). The study workflow is illustrated in Figure 1.

### Animal Model Preparation

Male C57BL/6 mice (body weight: 21–25 g) or Sprague-Dawley (SD) rats (body weight: ~220 g) were acclimatized for 3–5 days under standard laboratory conditions (12 h light/dark cycle, 22±1°C, 50±10% humidity) with ad libitum access to food and water. Prior to surgery, animals were fasted for 12 h with free access to water. Surgical Procedure:Anesthesia: Animals were anesthetized via intraperitoneal injection of 5% chloral hydrate (60 μL/10 g body weight). Preparation: Following loss of pedal reflex, animals were positioned in supine position on a heated surgical pad (37°C) and secured. Aseptic Technique: The abdominal area was shaved and disinfected with 10% povidone-iodine followed by 75% ethanol. Laparotomy: A 2 cm midline abdominal incision was made under sterile conditions using a scalpel. Cecal Ligation and Puncture (CLP):The cecum was exteriorized and isolated near the ileocecal valve;The distal 1/3 of the cecum was ligated with 3–0 silk suture;An 18-gauge needle was used to perforate the ligated cecum twice;Gentle pressure was applied to extrude a small amount of fecal content. Closure: The cecum was returned to the abdominal cavity, and the abdomen was closed in two layers (peritoneum and skin) with interrupted 4–0 silk sutures.

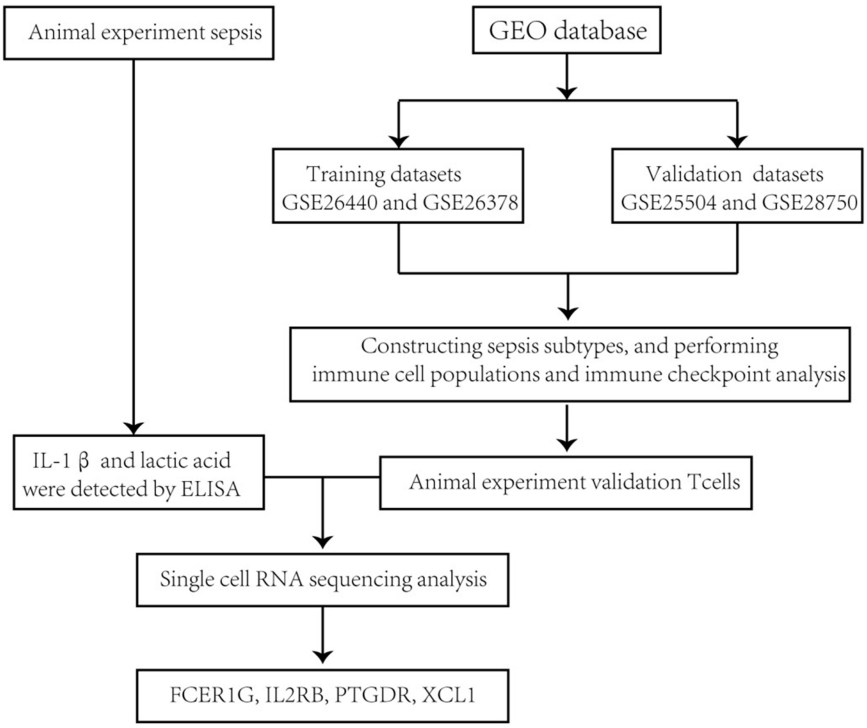


**Figure 1** Flow chart: ELISA detection of IL-1β, lactic acid, GEO data, differential expression of immune genes, GO, KEGG, PPI analysis, single-cell RNA sequencing analysis.

## Sample Collection

Following the successful establishment of the septicemia model via cecal ligation and puncture (CLP), blood and liver tissue samples were collected from both the control and CLP groups for subsequent analyses. Blood Sample Collection: At the designated time points post-CLP (48 hours), Group-wise euthanasia was performed by cervical dislocation, and whole blood was drawn via retro-orbital bleeding. Blood samples were centrifuged at 3000 × g for 15 min at 4°C to isolate serum/plasma, which was aliquoted and stored at −80°C until further analysis.

Liver Tissue Harvesting:After blood collection, mice in all groups were euthanized by cervical dislocation, and liver tissues were rapidly excised. For histopathological analysis.

## Enzyme-Linked Immunosorbent Assay (ELISA) for Cytokines

The concentrations of interleukin-1β (IL-1β) and lactate in mouse samples were quantified using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Meimian Biotechnology, Jiangsu, China). Specifically, IL-1β levels were measured using the Mouse IL-1β ELISA Kit (Catalog No. MM-0766M2), while lactate concentrations were determined using the Mouse Lactic Acid ELISA Kit (Catalog No. MM-0040M2). Following the manufacturer's protocol, absorbance (optical density, OD) was measured at 450 nm using a microplate reader, with blank wells serving as the zero reference. All readings were completed within 15 min after adding the stop solution to ensure assay accuracy.

## Bioinformatics Analysis of Differentially Expressed Immune-Related Genes

The gene expression datasets (GSE26440, GSE26378, GSE25504, and GSE28750) were obtained from the Gene Expression Omnibus (GEO) database, while immune-related gene sets were extracted from the ImmPort database. Raw microarray data were preprocessed by background correction using a normal-exponential convolution model, followed by normalization. Probe IDs were annotated to gene symbols using the UNIPROT database. To identify differentially expressed immune-related genes (DEIRGs), we performed comparative analysis between:Training sets (GSE26440 & GSE26378) and ImmPort data;Validation sets (GSE25504 & GSE28750) and ImmPort data. Differential expression analysis was conducted with a threshold of $|logFC| > 1$ and adjusted p-value (adj. p) $< 0.05$ to ensure

statistical significance. Overlapping genes across datasets were excluded, resulting in 19 unique DEIRGs for further functional and pathway enrichment analyses.

## Functional Enrichment Analysis of Differentially Expressed Immune-Related Genes

Functional annotation and pathway enrichment analysis were performed on the 19 differentially expressed immune-related genes (DEIRGs) using an integrated bioinformatics approach. GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analyses were conducted through the DAVID database (version 6.8). To ensure comprehensive functional characterization, we further employed Metascape and WebGestalt databases for complementary enrichment analysis. The GO analysis systematically evaluated gene functions across three domains; Biological Processes (BP), identifying key immune-related pathways;Cellular Components (CC), determining subcellular localization patterns;Molecular Functions (MF), characterizing biochemical activities. KEGG pathway analysis was employed to elucidate the higher-order functional networks and systemic interactions of these genes at both genomic and molecular levels. This approach enabled the identification of critical pathways involved in immune regulation and cellular homeostasis. All enrichment analyses were performed with a significance threshold of $p < 0.05$, with false discovery rate (FDR) correction for multiple testing. The integrated results from these complementary databases provided a robust framework for understanding the complex biological roles of the identified DEIRGs in immune system regulation and disease pathogenesis.

## Protein-Protein Interaction Network Analysis

To investigate the functional relationships among the identified differentially expressed immune-related genes (DEIRGs), we conducted a comprehensive protein-protein interaction (PPI) network analysis. The 19 DEIRGs were submitted to the STRING database (version 11.5; https://string-db.org/) using the following parameters:Organism: Homo sapiens (taxon ID 9606);Minimum required interaction score: 700 (high confidence);Active interaction sources: all available (including textmining, experiments, databases, co-expression, neighborhood, gene fusion, and co-occurrence). The resulting PPI network was further analyzed and visualized using Metascape to identify:Key hub proteins based on betweenness centrality and degree scores;Functionally clustered modules within the network;Enriched biological pathways among interacting proteins. Network topological parameters were calculated to assess the connectivity and biological relevance of the identified interactions. Only statistically significant interactions ($p < 0.05$) were retained for downstream analysis. This integrated approach allowed us to elucidate potential functional modules and core regulatory proteins within the immune-related gene network.

## Flow Cytometric Analysis of Immune Cell Populations

Twenty-four hours after surgery, mice from both the control group and sepsis model group (CLP) were euthanized, and whole blood samples were collected via cardiac puncture. Blood was immediately mixed with EDTA anticoagulant to prevent clotting. Lymphocyte Isolation:Blood samples were layered over lymphocyte separation medium (Ficoll-Paque PLUS, GE Healthcare) and centrifuged at 400 × g for 30 min at room temperature. The peripheral blood mononuclear cell (PBMC) layer was carefully aspirated, washed twice with PBS (pH 7.4), and resuspended in FACS buffer (PBS + 1% FBS). Flow Cytometry Staining & Analysis:T cells (Bioss Cat# bsm-30149A, PE, 1:150);B cells (Bioss Cat# bsm-30156A, FITC, 1:50);NK cells (Bioss Cat# bsm-30177M, PE, 1:100);Macrophages (Bioss Cat# bsm-30277A, PE, 1:50). After 30 min incubation at 4°C in the dark, cells were washed and analyzed using a NovoCyte flow cytometer (Model: Novocyte 2040R, Agilent). Data acquisition was performed using NovoExpress software (v1.6.1), and analysis was conducted with FlowJo (v10.8.1). All experimental procedures were approved by the Animal Care and Use Committee of Zhujiang Hospital, Southern Medical University and performed in accordance with relevant guidelines and regulations. Guidelines for the Welfare and Ethical Review of Laboratory Animals (GB/T 35892–2018). Animal Ethics Committee Approval Number: IAEC-K-240228-02. Laboratory Animal Use License Number: SYXK (Yue) 2023–0318. Antibody concentrations and staining conditions were optimized through preliminary titration experiments to ensure optimal signal-to-noise ratios.

## Immune Infiltration Analysis of Sepsis-Associated Genes

Database and Analytical Approach:We employed the PanglaoDB database (https://panglaodb.se/index.html), a comprehensive online resource for single-cell RNA sequencing data analysis, to investigate the immune cell specificity of our 19 identified differentially expressed immune-related genes (DEIRGs). This database integrates scRNA-seq data from multiple tissues and cell types, enabling systematic evaluation of gene expression patterns at single-cell resolution. Analysis Pipeline:Data Input and Processing:Uploaded the 19 DEIRGs identified from GEO datasets (GSE26440, GSE26378, GSE25504, and GSE28750);Applied the database's standardized normalization and quality control procedures;Restricted analysis to immune cell populations, with particular focus on T cell subsets. T Cell Association Analysis:Evaluated gene expression specificity across:Naïve T cells,Memory T cells,Regulatory T cells (Tregs), Cytotoxic T cells. Calculated expression scores using the database's proprietary algorithms;Applied statistical thresholds ($p < 0.05$, expression level > 1 TPM) for significance. Validation and Integration:Cross-referenced results with independent scRNA-seq datasets;Performed co-expression network analysis;Validated findings against established T cell marker genes.

## Histopathological Analysis by Hematoxylin-Eosin (H&E) Staining

Liver tissues were collected 24 hours post-modeling from both septic (CLP) and sham-operated control mice. After fixation in 4% paraformaldehyde for 24 hours, tissues were processed through graded ethanol series, embedded in paraffin, and sectioned at 4 μm thickness. For H&E staining, sections were deparaffinized in xylene and rehydrated through graded alcohols, then stained with hematoxylin solution (Servicebio, G1004) for 5 minutes, differentiated in hematoxylin differentiation buffer (Servicebio, G1039-500ML) for 30 seconds, and rinsed in hematoxylin bluing buffer (Servicebio, G1040-500ML) to restore nuclear blue staining. Cytoplasmic counterstaining was performed using eosin alcohol solution (Servicebio, G1001) for 2 minutes. After dehydration, sections were mounted with neutral balsam. Histopathological evaluation was performed by two blinded pathologists, assessing: (1) neutrophil infiltration density per high-power field (400×), (2) degree of hepatic architecture disruption, (3) area of focal necrosis, and (4) severity of sinusoidal congestion. Five random fields per section were analyzed using ImageJ software, with inter-observer variability maintained below 10%.

Note: All staining reagents were from Servicebio (Wuhan, China), with lot numbers recorded for quality control purposes.

## Immunohistochemical (IHC) Analysis of IL-1β and TNF-α Expression

Liver tissue sections (4-μm thick, paraffin-embedded) were subjected to antigen retrieval by heating in citrate buffer (pH 6.0) at 95°C for 20 min. Endogenous peroxidase activity was quenched with 3% hydrogen peroxide for 15 min at room temperature. After blocking non-specific binding sites with 5% bovine serum albumin (BSA) for 30 min, sections were incubated overnight at 4°C with the following primary antibodies: rabbit anti-IL-1β (1:200; Abcam, ab9722) and rabbit anti-TNF-α (1:150; Abcam, ab6671). Immunodetection was performed using the UltraSensitive S-P Super-Sensitive Kit (Rabbit; Maixin Biotech, KIT-9706) according to the manufacturer's instructions. Briefly, sections were incubated with HRP-conjugated secondary antibody (1:500) for 1 h at room temperature, followed by visualization with 3,3′-diaminobenzidine (DAB) and counterstaining with hematoxylin. Appropriate negative controls (primary antibody omitted) were processed in parallel for each staining batch. Staining was quantified using ImageJ with IHC Profiler plugin, calculating: (1) percentage of positive cells, (2) staining intensity (0–3 scale), and (3) H-score (intensity × percentage) across five random fields per section. Data are presented as mean ± SEM, with statistical significance ($p < 0.05$) determined by unpaired *t*-test.

## Statistical Analysis

All data are expressed as mean ± standard error of the mean (SEM). Statistical analyses were performed using SPSS 22.0 software (IBM, USA). Differences between groups were assessed by independent samples *t*-test (unpaired, two-tailed). A p-value < 0.05 was considered statistically significant.

Sepsis is characterized by systemic inflammation and metabolic disturbances, including hyperlactatemia. To investigate sepsis-induced inflammatory responses, we established a cecal ligation and puncture (CLP)-induced murine sepsis model with concurrent liver injury.

# Result

## Sepsis Provokes Systemic Inflammation and Metabolic Imbalance

Blood samples were collected at 48 h post-CLP for ELISA analysis of the pro-inflammatory cytokine IL-1β and circulating lactate levels, key indicators of sepsis-associated inflammation and metabolic dysfunction, respectively. IL-1β levels were significantly elevated in the CLP group compared to the sham-operated controls ($P < 0.01$), confirming a robust systemic inflammatory response (Figure 2B). Similarly, lactate accumulation was markedly higher in septic mice ($P < 0.01$), indicating hyperlactatemia, a hallmark of sepsis-induced metabolic dysregulation (Figure 2C). These findings demonstrate the successful establishment of the CLP-induced sepsis model (Figure 2A) and validate that sepsis triggers both inflammatory activation and metabolic disturbances, including hyperlactatemia.

## Identification of Differentially Expressed Immune-Related Genes (DEIRGs)

Volcano plot analysis of the four datasets revealed significant differentially expressed genes (DEGs) associated with sepsis (Figure 3A–D). Cross-referencing the training sets (GSE26440 & GSE26378) with immune-related gene databases yielded 17 overlapping immune-related DEGs (Figure 3E). Similarly, the validation sets (GSE25504 & GSE28750) identified 3 intersecting immune-related DEGs (Figure 3F). After removing redundant entries, a total of 19 unique immune-related DEGs were identified.

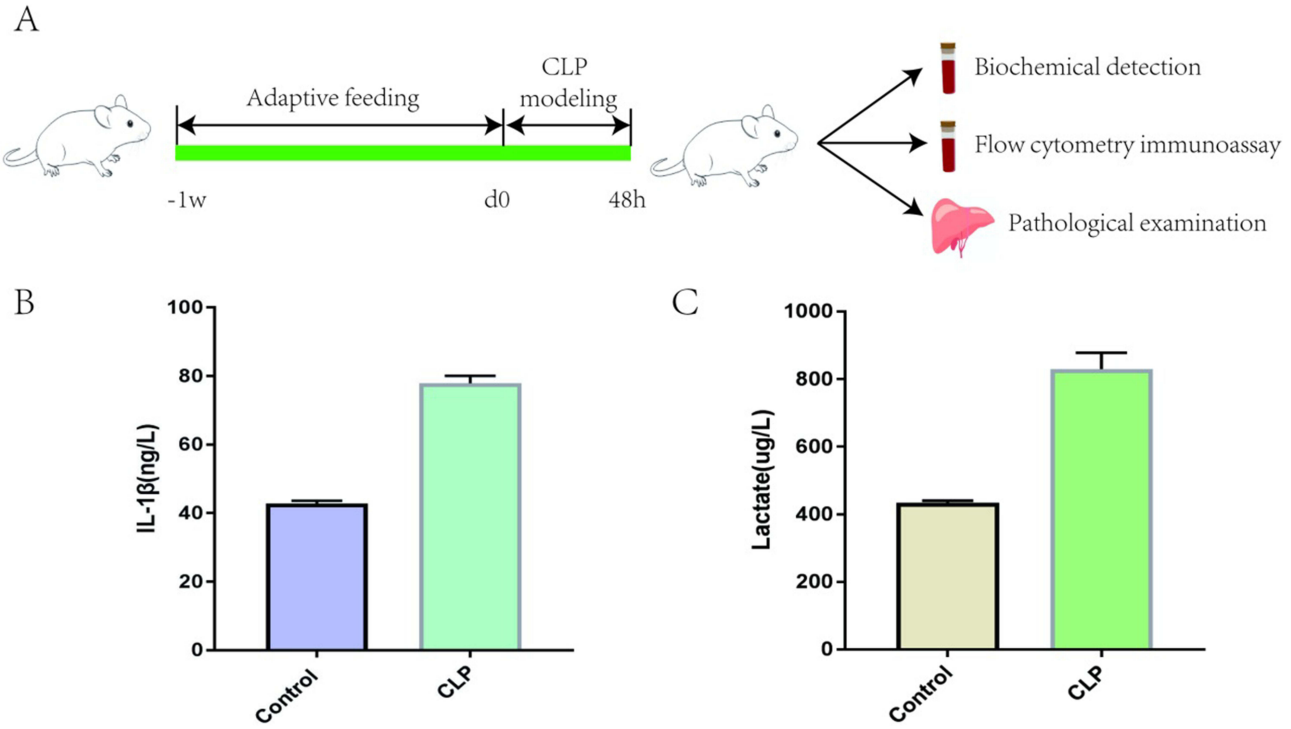


**Figure 2** Establishment of the CLP-induced sepsis model in mice and its induction of systemic inflammatory response and metabolic disorders. Control: negative control group, CLP: sepsis model group. (**A**) Schematic diagram of the construction of the mouse CLP model. (**B**) The expression levels of IL-1βin mice sepsis were detected by Elisa. (**C**) The expression levels of lactic acid in mice sepsis were detected by Elisa.

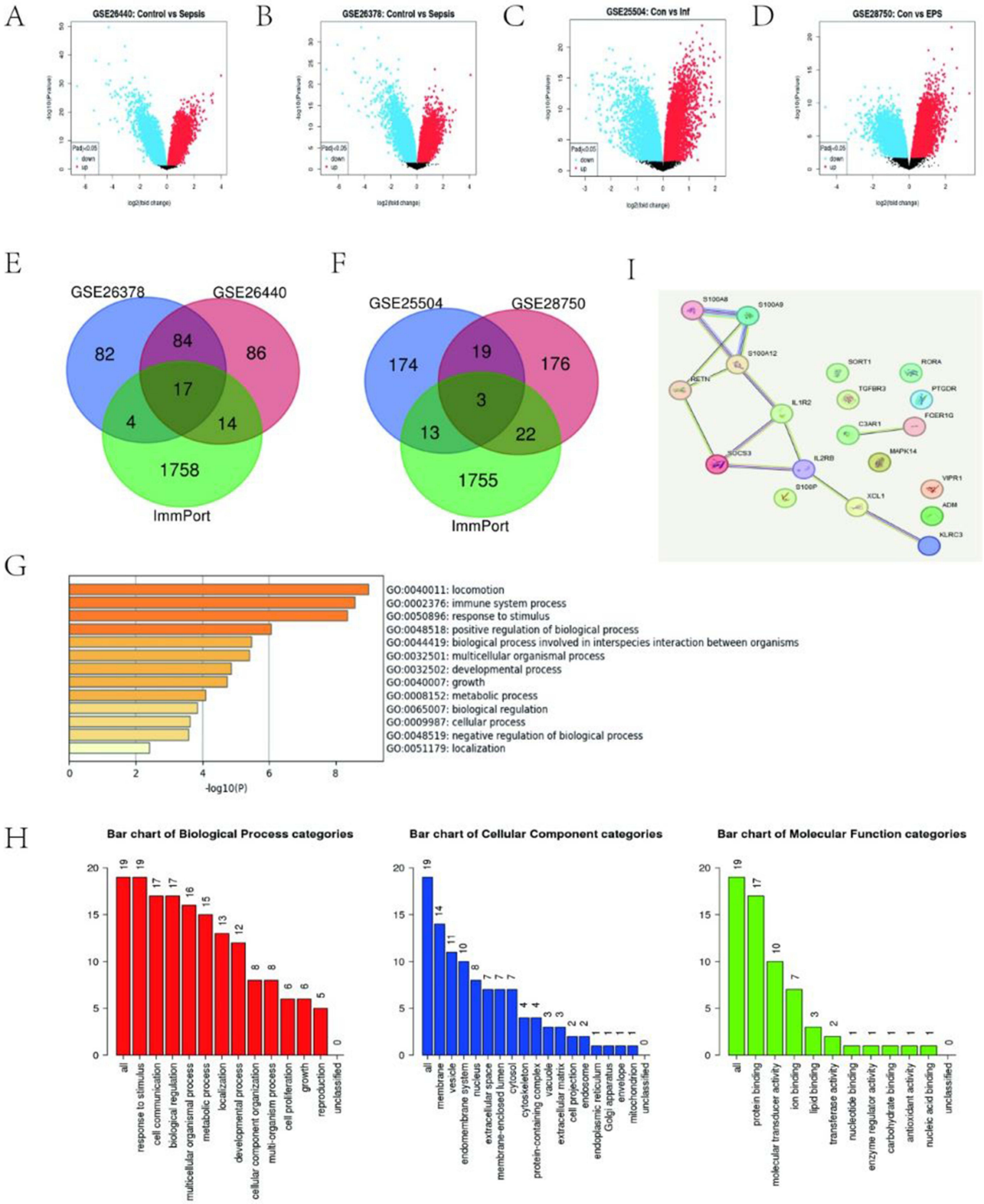


**Figure 3** Identifcation of DEIRGs in sepsis. (**A**) Volcano map of GSE26440 differentially expressed genes. (**B**) Volcano map of GSE26378 differentially expressed genes. (**C**) Volcano map of GSE25504 differentially expressed genes. (**D**) Volcano map of GSE28750 differentially expressed genes. (**E**) Venn graph screening DEIRGs of GSE26440 and GSE26378. (**F**) Venn graph screening DEIRGs of GSE25504 and GSE28750. (**G**) Pathway results of 19 significantly differentially expressed genes. (**H**) Gene annotations of 19 significantly differentially expressed genes. (**I**) PPI protein co-expression network.


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## Functional Enrichment and Pathway Analysis

Gene Ontology (GO) and KEGG pathway analysis (via Metascape & WebGestalt) demonstrated that these DEGs were significantly enriched in immune response pathways, further supporting the critical role of immune dysregulation in sepsis (Figure 3G and H).

## Protein-Protein Interaction (PPI) Network Analysis

PPI network analysis (using STRING or similar tools) revealed:19 nodes (genes) and 13 interaction edges;Average node degree: 1.37;Average local clustering coefficient: 0.333;PPI enrichment p-value: $3.35 \times 10^{-6}$, indicating statistically significant biological interactions (Figure 3I).

These findings strongly suggest that sepsis pathogenesis is closely linked to immune regulatory mechanisms, with identified DEGs playing potential roles in immune dysfunction.

## T-Cell Immunomodulation Plays a Critical Role in Sepsis-Induced Inflammatory Response

To characterize the immune cell populations involved in sepsis pathogenesis, we performed flow cytometry analysis on a CLP-induced murine sepsis model. Key immune cell subsets were identified using the following surface markers:B cells (CD19+),T cells (CD3+),NK cells (NK1.1+),Macrophages (F4/80+). After background signal correction, we observed:T cells (CD3+) exhibited the most significant response, with:65.87% frequency in CLP group vs 63.85% in sham controls (Figure 4A–D). Other immune cell populations (B cells, NK cells, macrophages) showed no statistically significant changes. The selective T-cell predominance in septic mice suggests that:T-cell-mediated immunity is a key driver of sepsis-induced inflammation;T-cell activation or dysregulation may contribute to the hyperinflammatory state in sepsis.

These findings align with emerging evidence that T-cell immunomodulation critically influences sepsis outcomes, potentially offering novel therapeutic targets.

## Identification of T Cell-Associated Genes in Septic Liver Injury

To elucidate the molecular mechanisms underlying T cell-mediated immune responses in sepsis-induced liver injury, we conducted single-cell RNA sequencing (scRNA-seq) analysis using the PanglaoDB database. The enrichment of these genes suggests their involvement in T cell-mediated immune regulatory pathways during sepsis-induced liver injury (Figure 4E–H).

## Sepsis Triggers Liver Injury and Inflammatory Activation

To characterize sepsis-induced hepatic pathology, we established a cecal ligation and puncture (CLP)-mediated murine sepsis model and performed comprehensive analyses of liver tissue. Histopathological Evaluation Sham-operated controls:Maintained normal hepatic architecture. Intact lobular organization. No evidence of cellular damage.CLP-induced sepsis:Demonstrated focal necroinflammation. Disrupted parenchymal structure. Hepatocyte swelling and inflammatory cell infiltration (Figure 5A). Inflammatory Cytokine Profiling:Immunohistochemical analysis revealed significant upregulation of pro-inflammatory mediators:IL-1β expression: Markedly increased in septic livers vs controls (Figure 5B). TNF-α expression: Substantially elevated in model group (Figure 5C).

# Discussion

Our study provides comprehensive insights into the immunopathological mechanisms underlying sepsis-induced liver injury and identifies potential therapeutic targets through integrated bioinformatics and experimental approaches. The pathological findings from our CLP-induced sepsis model demonstrated significant hepatic inflammation, characterized by neutrophil infiltration and elevated pro-inflammatory cytokines (IL-1β and TNF-α), consistent with previous reports of sepsis-associated liver injury.[9] Notably, our flow cytometry analysis revealed substantial alterations in T cell populations, suggesting their crucial role in sepsis immunopathology. These findings align with emerging evidence that T cell subsets

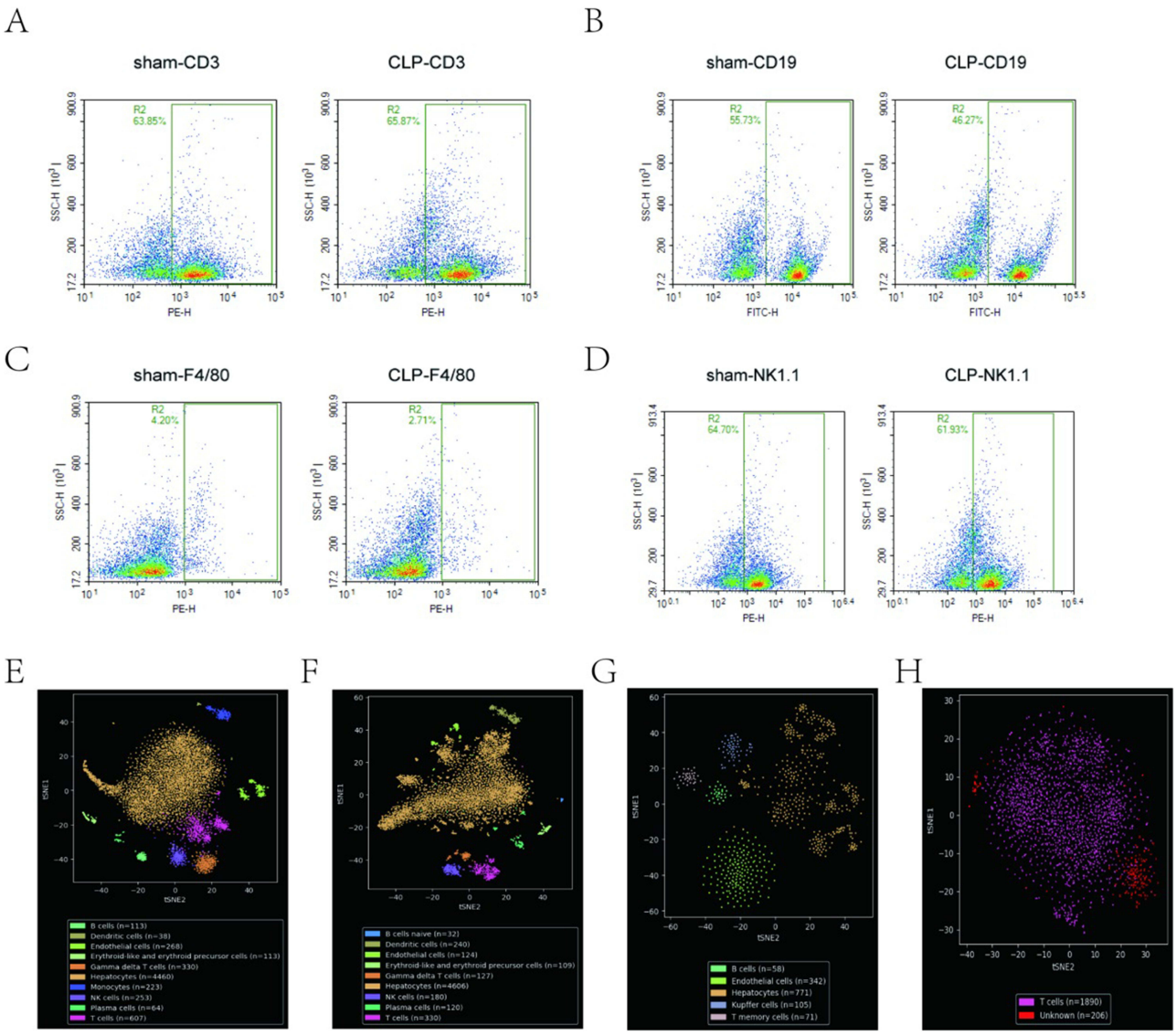


**Figure 4** Immune markers were used to detect the type of immune cells in mice with sepsis and liver immune cell typing by single cell RNA sequencing. (**A**) Flow cytometry results of immune T lymphocytes (CD3+). (**B**) Flow cytometry results of immune B lymphocytes (CD19+). (**C**) Flow cytometry results of immune Macrophages (F4/80+). (**D**) Flow cytometry results of immune NK cells (NK1.1+). (**E**) Single-cell sequencing screened out the T-cell-related immune gene FCER1G in liver injury caused by sepsis. (**F**) Single-cell sequencing screened out the T-cell-related immune gene IL2RB in liver injury caused by sepsis. (**G**) Single-cell sequencing screened out the T-cell-related immune gene PTGDR in liver injury caused by sepsis. (**H**) Single-cell sequencing screened out the T-cell-related immune gene XCL1 in liver injury caused by sepsis.

participate in both pro-inflammatory and regulatory responses during sepsis.[10] Through systematic bioinformatics analysis of the GEO dataset, we identified 19 differentially expressed immune-related genes (DEIRGs). Combined with flow cytometry immunocytometry and single-cell sequencing analysis, the four core genes (FCER1G, IL2RB, PTGDR and XCL1) were further emphasized. They may be the key regulatory factors of immune dysregulation in sepsis.

Bioinformatics analysis of 19 significantly differentially expressed immune-related genes revealed distinct interaction patterns within the PPI network. Notably, FCER1G (Fc epsilon receptor Ig), a key mediator of immune receptor signaling, showed no direct association with PTGDR (Prostaglandin D2 Receptor). However, XCL1 and IL2RB exhibited a functional linkage, suggesting a synergistic regulatory role in liver immunity. FCER1G exerts dual effects in T cell-mediated hepatic immunity by activating downstream pathways (NF-κB, MAPK, PI3K/AKT, IL-6/STAT3, IL-12/STAT4) through its ITAM domain, promoting antiviral defense while suppressing tumor progression.[11–14] Conversely, PTGDR isoforms (DP1/DP2) differentially modulate liver pathology—DP2 exacerbates inflammation via NF-κB-driven


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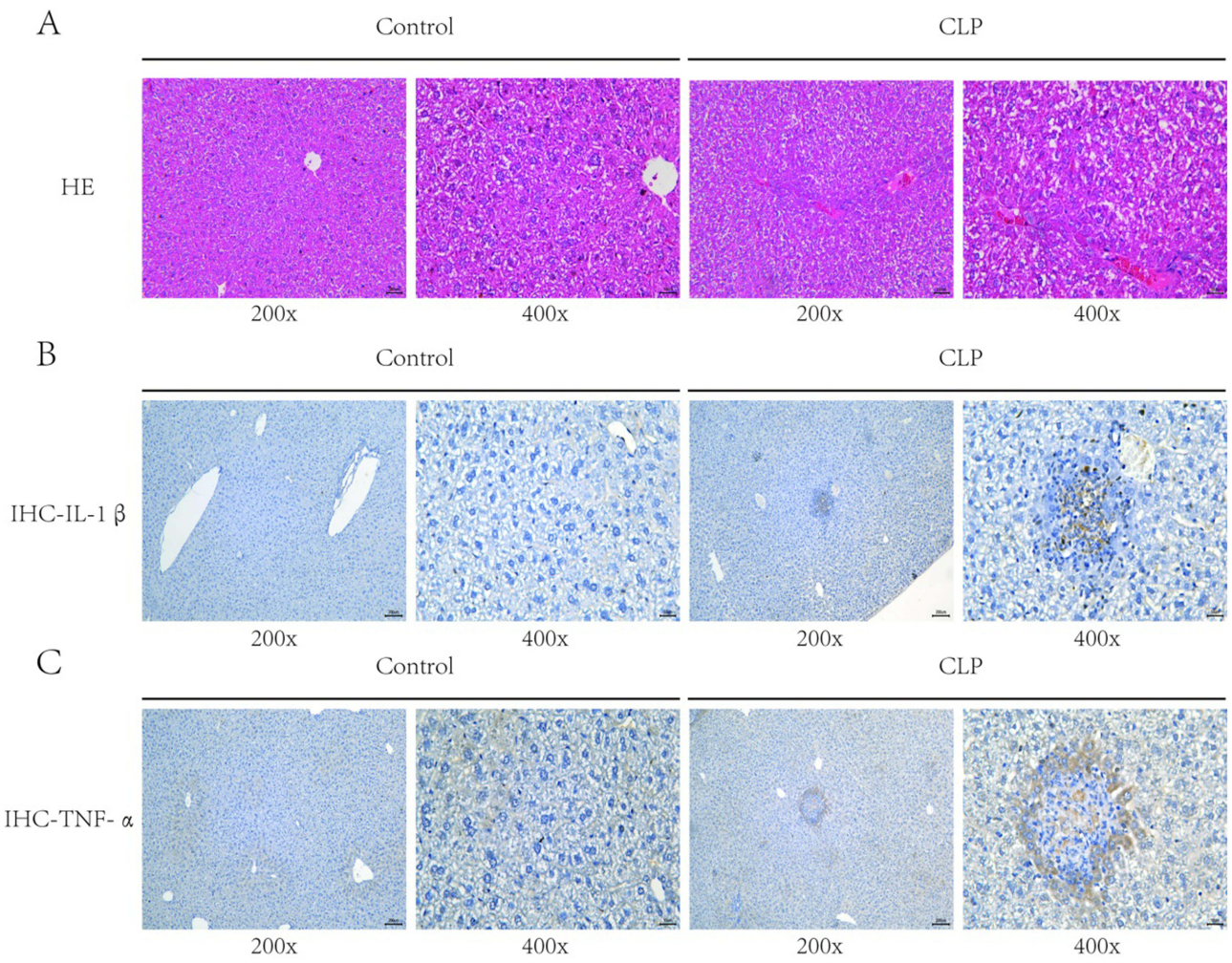


**Figure 5** HE and ICH detection. (**A**) HE detected the immune infiltration of liver cells in the sepsis experimental group and the control group. (**B**) ICH detected the expression level of IL-1β in the sepsis experimental group and the control group. (**C**) ICH detected the expression level of TNF-α in the sepsis experimental group and the control group.

Th17 polarization, whereas DP1 enhances Treg-mediated hepatoprotection via cAMP-PKA signaling.[15–17] These opposing mechanisms highlight their potential as therapeutic targets in liver diseases, including sepsis-associated injury.

Furthermore, IL2RB (a shared subunit of IL-2/IL-15 receptors) and XCL1 (a C-type chemokine) form a positive feedback loop:[18,19] IL2RB activates STAT5 via the IL-2/IL-15-JAK/STAT5 axis,[20] upregulating XCL1 secretion from T/NK cells.[21] XCL1-XCR1 signaling then recruits cDC1 dendritic cells, amplifying antigen presentation and CTL responses.[22] Activated cDC1 further secretes IL-15, reinforcing IL2RB signaling—a synergistic circuit enhancing antiviral and antitumor immunity.[23] This crosstalk underscores their pivotal role in fine-tuning T cell-mediated hepatic immune responses, offering novel targets for precision immunotherapy.

While our study provides valuable insights, several limitations should be acknowledged. The sample size for experimental validation was relatively small, and our findings require confirmation in larger clinical cohorts. Additionally, the precise mechanisms through which these candidate genes influence sepsis progression warrant further investigation using targeted genetic approaches. Future research directions should include:Functional validation of candidate genes using knockout/knockin models. Longitudinal studies to assess dynamic changes in gene expression during sepsis progression. Development of targeted therapeutic strategies based on identified molecular pathways.

In conclusion, our integrated approach combining bioinformatics analysis with experimental validation has identified FCER1G, IL2RB, PTGDR, and XCL1 as potential key regulators of immune dysregulation in sepsis. These findings

provide a foundation for developing novel diagnostic markers and targeted therapies, potentially improving outcomes for this devastating condition. The convergence of these genes on T cell regulation pathways highlights the central role of adaptive immunity in sepsis pathogenesis and suggests promising avenues for immunotherapy development.

## Conclusion

In summary, this study shows that FCER1G, IL2RB, PTGDR, XCL1 play a crucial role in the progression of liver injury in children with sepsis and are closely related to immune cell populations.

Immune checkpoint regulation is a biomarker with important diagnostic value and a potential immunotherapy.

## Data Sharing Statement

Datasets analysed in this study are available from GEO database (http://www.ncbi.nlm.nih.gov/geo/); Immune-related genes were derived from ImmPort databases (). GO gene annotation and KEGG pathway analysis from DAVID database (https://metascape.org/gp/index.html#/main/) and webgestalt database (https://www.webgestalt.org/option.php), metascape database (https://metascape.org/gp/index.html#/main/step1); PPI protein interaction and co-expression network was derived from string database (https://cn.string-db.org/cgi/input). Single cell RNA sequencing analysis data from panglaodb database (https://panglaodb.se/index.html).

## Ethics Approval and Consent to Participate

This study has been reviewed and approved by the Ethics Committee of Zhujiang Hospital of Southern Medical University. The study strictly adheres to the regulations of the Ethics Committee to ensure that the rights and interests of all participants are fully protected during the research process.

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## Disclosure

The authors declare that they have no competing interests in this work.

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