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ORIGINAL RESEARCH

Exploring the Role of Peroxisome-Related Processes and Key Marker Genes in Sepsis: Insights Into Immune Dynamics and Therapeutic Potential

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Background: Peroxisomes are vital organelles involved in metabolic processes such as fatty acid metabolism and reactive oxygen species detoxification. Their role in sepsis, characterized by systemic inflammation and organ dysfunction, is not well understood. This study focuses on pediatric sepsis, utilizing gene expression profiles from pediatric sepsis patients and healthy controls.

Methods: We analyzed differentially expressed peroxisome-related genes (DPGs) using the GSE26378 dataset. ssGSEA assessed peroxisome pathway activity, immune cell infiltration, and immune-related pathways, examining their relationships. Key marker genes were identified using LASSO, RF, and Logistic Regression. A nomogram was constructed to predict sepsis risk based on these markers. An in vivo animal model of LPS-induced sepsis was also utilized to validate the expression of key marker genes (EPHX2 and IDH1).

Results: Sixteen DPGs were identified in sepsis. Peroxisome pathway activity was significantly increased in the sepsis group, positively correlating with neutrophils and macrophages, and negatively with NK cells. In sepsis, pathways related to immunity, inflammation, and apoptosis were activated, with a strong positive correlation between peroxisome activity and these pathways. Machine learning algorithms revealed key marker genes, EPHX2 and IDH1, validated through the GSE13904 and GSE26440 datasets, and LPS-induced animal experiments.

Conclusion: Our findings underscore the significant role of peroxisome-related processes in sepsis, particularly their interaction with immune dynamics and pathways. The identified marker genes may serve as potential therapeutic targets, by linking immune infiltration to peroxisomal gene expression, enhancing our understanding of sepsis pathophysiology and improving clinical outcomes.

Keywords: sepsis, peroxisome, immune cell infiltration, RNA sequencing, biomarker identification

Introduction

Sepsis is a life-threatening condition characterized by a dysregulated host response to infection, leading to systemic inflammation, multiple organ dysfunction, and high mortality rates.[1,2] Despite advances in critical care, the pathophysiological mechanisms underlying sepsis remain incompletely understood, posing significant challenges to early diagnosis and effective treatment.[3,4] Recent studies have pointed towards the crucial role of metabolic pathways and immune responses in the progression of sepsis, yet the specific contributions of various cellular organelles and their related processes are still being elucidated.

Peroxisomes are essential organelles involved in fatty acid β-oxidation, reactive oxygen species (ROS) detoxification, and plasmalogen biosynthesis.[5–7] These processes help maintain cellular homeostasis and protect cells from oxidative damage. The involvement of peroxisomes in immune regulation and their response during systemic inflammatory conditions like sepsis is a relatively recent area of interest. However, the role of peroxisome-related processes in sepsis has not been adequately explored, particularly in pediatric cohorts, where immune responses differ from adults. Previous

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research has established the importance of metabolic reprogramming and immune cell function in sepsis.[8,9] Studies have shown alterations in lipid metabolism and increased oxidative stress in septic patients, implicating peroxisomes in the pathophysiology of sepsis.[10,11] For example, elevated oxidative stress has been associated with impaired immune cell function and heightened inflammatory responses, contributing to the severity of sepsis.[12] Moreover, peroxisomal enzymes such as catalase and superoxide dismutase play critical roles in mitigating ROS, which are often elevated during sepsis.[13,14] Despite these insights, few studies have specifically focused on the differential expression of peroxisome-related genes (DPGs) and their impact on immune cell dynamics in sepsis. Most existing research has been limited to animal models, leaving a significant gap in our understanding of how peroxisomal processes affect patients differently. This highlights the need for studies that specifically address how peroxisomal processes influence sepsis outcomes in pediatric patients.

The high morbidity and mortality associated with sepsis necessitate the identification of novel biomarkers and therapeutic targets. Understanding the interactions between peroxisome-related processes and immune responses could offer new avenues for early diagnosis and targeted therapies. Given the unique metabolic and immune characteristics of patients, it is particularly important to investigate how peroxisomal function influences sepsis outcomes in children, where immune responses may differ from those in adults. This study aims to address this gap by performing a detailed bioinformatic analysis of DPGs in sepsis, utilizing advanced computational techniques to uncover potential biomarkers and therapeutic targets. By linking peroxisome activity with immune cell infiltration and immune-related pathways, we seek to provide a comprehensive overview of how peroxisomal processes contribute to the pathophysiology of sepsis.

Methods

Data Collection and Preprocessing

We sourced sepsis expression data from the Gene Expression Omnibus (GEO) database (accessed on June 19, 2024; https://www.ncbi.nlm.nih.gov/geo/), specifically datasets GSE26378 (21 healthy controls and 82 septic samples), GSE26440 (32 healthy controls and 98 septic samples), and GSE13904 (18 healthy controls and 158 septic samples) as the validation cohort (Table S1). Inclusion criteria for samples required: (1) cohort size ≥80 patients, (2) blood-derived samples, and (3) age-matched controls without recent fever (within 2 weeks), anti-inflammatory drug use (within 2 weeks), or history of inflammatory diseases. The demographic and clinical features of sepsis cases from the GSE26440 and GSE26378 datasets are summarized in Table S2. Annotation files for GPL570, along with the matrix files from both datasets, were obtained from the GEO database. Quality control of matrix files included outlier detection using RLE plot, with no samples excluded post-QC. Expression values were generated using the Affy package (v1.86.0) with background correction and normalization via the gcrma package (v2.80.0). After preprocessing, the final dataset included 20,549 genes.

Differential Expression Analysis

Peroxisome-related genes were curated from the "KEGG_Peroxisome" and "Hallmark_Peroxisome" gene sets in the Molecular Signatures Database (MsigDB), yielding 88 peroxisome-related genes for analysis. Differential expression analysis was conducted using the limma package (v3.52.2) on 20,549 genes across all samples. The threshold for significance was set at an adjusted p-value < 0.05 and $|\log2 \text{ fold change (log2FC)}| > 0.5$, a cutoff supported by prior studies of sepsis-related transcriptomic changes.[15] Among the analyzed genes, 16 peroxisome-related genes exhibited significant differential expression. Visualization of results included: Volcano plots generated using the ggplot2 package (v3.4.4); heatmaps created with the ComplexHeatmap package (v2.13.1); correlation networks constructed using igraph (v1.4.1) and ggraph (v2.1.0) packages.

Single-Sample Gene Set Enrichment Analysis (ssGSEA)

To evaluate the peroxisome pathway's activity, we employed ssGSEA, using R's GSVA package, generating pathway scores for each sample. Comparison of ssGSEA scores between sepsis and control groups allowed us to assess peroxisome pathway activity. For immune cell infiltration analysis, we utilized marker gene sets of 24 immune cell types from Bindea et al.[16] For immune-associated pathway activity, gene sets were obtained from the ImmPort database

(http://www.immport.org). The correlation between peroxisome pathway scores and immune cell infiltration/immune pathway scores was calculated using Spearman correlation, with p-values adjusted for multiple comparisons using the Benjamini-Hochberg method.

Enrichment Analyses

Gene Ontology (GO) functional annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed on peroxisome-related genes using the clusterProfiler and enrichplot packages. GO analysis covered all three ontology domains: Biological Process (BP), Cellular Component (CC), and Molecular Function (MF). Statistical significance was defined as an adjusted p-value < 0.05 (Benjamini-Hochberg correction).

Gene Set Variation Analysis (GSVA)

The "hallmark" gene sets (50 pathways) from the MSigDB database were analyzed using the Limma and GSVA packages to compare pathway activity between control and sepsis groups. Differential activity was assessed with Limma's linear models, applying an adjusted p-value threshold of < 0.05. Results are visualized in box plots.

Machine Learning for Key Gene Identification

To identify potential marker genes from DPGs associated with sepsis, we employed three machine learning algorithms: (1) LASSO regression, where the optimal lambda value was selected via 10-fold cross-validation; (2) Random Forest, with hyperparameter tuning through grid search to optimize tree depth and the number of trees; and (3) Logistic Regression, incorporating L2 regularization to prevent overfitting. Model performance was evaluated using 5-fold cross-validation, with metrics including AUC, accuracy, sensitivity, and specificity. Class imbalance was addressed via stratified sampling during cross-validation. The LASSO regression was optimized to determine the most effective lambda value. Meanwhile, the Random Forest algorithm assessed the importance of genes based on mean decrease accuracy, leading to the selection of the top 10 most significant genes. Genes consistently identified across all three methods were selected as key markers for sepsis.

Nomogram Development and Validation

A nomogram was developed based on the expression levels of EPHX2 and IDH1 using multivariable logistic regression with bootstrap resampling (1000 iterations). Model performance was assessed using AUC (with 95% confidence intervals), calibration plots (Hosmer-Lemeshow test), and decision curve analysis (DCA). Internal validation was conducted using the GSE26378 dataset, while external validation employed the GSE13904 dataset.

Animal Experiments

Male C57BL/6J mice, 8 weeks old, were obtained from the Laboratory Animal Center of Nanchang University. The sample size ($n = 12$ per group) was chosen based on previous studies demonstrating significant effects of LPS at this dose in similar experimental designs and our preliminary data, although a formal power calculation was not performed. The mice were housed in a standard animal care facility under controlled conditions (temperature: 22 ± 1°C, humidity: 55 ± 5%, 12:12 hour light-dark cycle) with free access to standard chow and water. Mice were randomly assigned to either the Control group or the Sepsis group ($n = 12$ per group) using a random number table generated prior to the start of the experiment. The Control group received an intraperitoneal injection of sterile saline, whereas the Sepsis group received an intraperitoneal injection of lipopolysaccharide (LPS) at a dose of 35 mg/kg (Sigma). Following LPS administration, mice were monitored every 15 minutes for the first hour, then hourly for a total of 6 hours, for signs of severe distress (eg, piloerection, lethargy, labored breathing, inability to access food/water). A predefined humane endpoint protocol was established, requiring euthanasia if mice exhibited severe respiratory distress, unresponsiveness to stimuli, or inability to remain upright. No animals reached these endpoints during the 1-hour experimental period prior to sample collection. All experimental procedures were conducted in accordance with the guidelines approved by the Ethics Committee of Nanchang People's Hospital (Approval No. 20240161). Blood specimens were obtained via retro-orbital puncture one hour following the administration of LPS. Personnel performing the blood collection, RNA isolation, cDNA

synthesis, and subsequent qRT-PCR analyses were blinded to the group allocation of the samples (Control vs Sepsis). Total RNA was isolated from the blood samples using TRIzol reagent (ThermoFisher Scientific, USA) following the manufacturer's instructions. The extracted RNA was then used to synthesize complementary DNA (cDNA) employing the cDNA Synthesis Kit (ThermoFisher Scientific, USA). Quantitative real-time PCR (qRT-PCR) was performed using the SYBR GREEN PCR Master Mix on a Real-Time PCR system (Applied Biosystems 7500). For each target gene (EPHX2, IDH1) and the reference gene (β-actin), qRT-PCR reactions were run in technical duplicate for each of the 12 biological replicates (individual mice) per group. Amplification efficiency for each primer pair was validated prior to the main experiment and fell within the acceptable range of 90–110%. Furthermore, melt curve analysis was performed at the end of each qRT-PCR run to confirm the specificity of amplification and the presence of a single PCR product. The relative mRNA expression levels of the target genes, EPHX2 and IDH1, were quantified using the $2^{-\Delta\Delta Ct}$ method.

Results

Identification of Differentially Expressed Peroxisome-Related Genes (DPGs) in Sepsis

Figure 1A presents a volcano plot summarizing the differential expression analysis between control and septic groups. A total of 2446 genes showed significant differential expression, with 1290 genes upregulated and 1156 genes downregulated in the sepsis group compared to the control group, as indicated by red and blue dots, respectively. Among these, 16 peroxisome-related genes were identified as differentially expressed. To investigate the relationships between these DPGs, the correlation network (Figure 1B) and heatmap (Figure 1C) were constructed. The results display strong correlations among the 16 genes. Box plots in Figure 1D provide a comparative analysis of gene expression levels

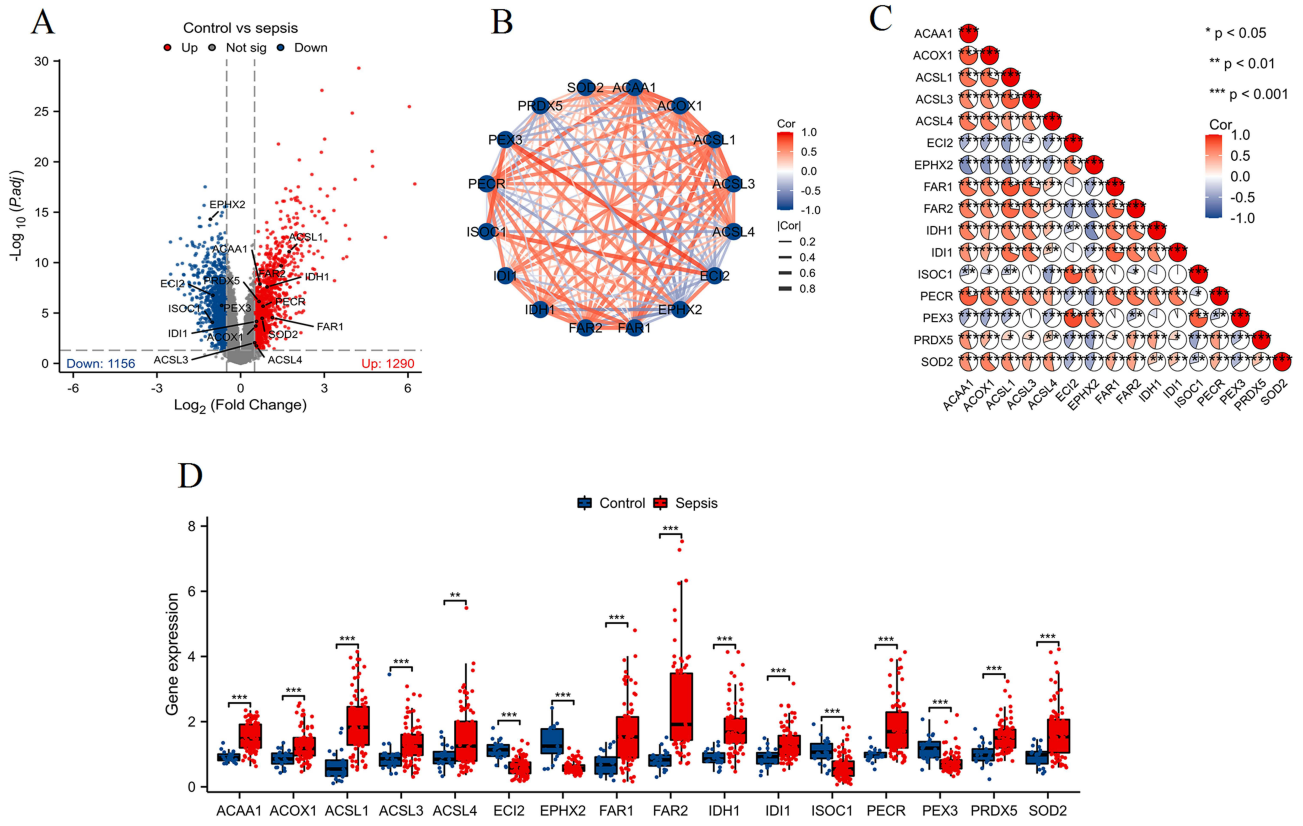

Figure 1 Analysis of differentially expressed peroxisome-related genes (DPGs) between sepsis and control groups. (**A**) Volcano plot displaying gene expression differences between control (n = 21) and sepsis (n = 82) groups. Red dots represent upregulated genes, blue dots represent downregulated genes, and grey dots indicate genes with no significant change. The 16 DPGs are labeled. (**B**) Correlation network of DPGs. Nodes represent genes, with edge thickness and color denoting the strength and direction of the Spearman correlation (positive in red, negative in blue, and thicker edges indicating stronger correlations). (**C**) Heatmap showing Spearman correlation coefficients among DPGs. Significant correlations are marked with * (p < 0.05), ** (p < 0.01), and *** (p < 0.001). (**D**) Box plots comparing gene expression levels of 16 DPGs between control (blue, n = 21) and sepsis (red, n = 82) groups. Statistical significance was assessed using the Wilcoxon rank-sum test, and p-values were adjusted for multiple comparisons using the Benjamini-Hochberg false discovery rate (FDR) method (** p < 0.01, *** p < 0.001).

between control and septic groups. Each box plot represents the expression distribution of an individual gene in both groups. Significant differences in expression levels were observed, with the sepsis group exhibiting markedly higher expression levels for most of the 16 peroxisome-related genes ($p < 0.01$), confirming their upregulation in response to sepsis. In addition, the functional enrichment analysis suggests that the DPGs in sepsis are primarily involved in fatty acid metabolism and closely associated with peroxisomal functions, revealing potential mechanisms by which these pathways may contribute to the pathophysiology of sepsis (Figure S1). In summary, these analyses identified and characterized 16 peroxisome-related genes with significantly altered expression in sepsis, providing insights into their potential roles in sepsis pathogenesis.

Assessment of Peroxisome Pathway Level and Its Relationship with Immune Cells

Figure 2A shows a box plot comparing the ssGSEA score of the peroxisome pathway between control and sepsis groups. The peroxisome pathway activity was significantly higher in the sepsis group compared to the control group ($p < 0.001$), indicating an upregulation of peroxisome-related activities during sepsis. In order to explore the relationship between peroxisome pathway activity and immune cell infiltration, we performed a correlation analysis. Figure 2B highlights the correlation coefficients between peroxisome score and various immune cell types. Neutrophils, macrophages, and Th17 cells exhibited a strong positive correlation with peroxisome scores. Conversely, several immune cell types, including NK CD56bright cells and NK cells, exhibited negative correlations with peroxisome score. Significant differences in

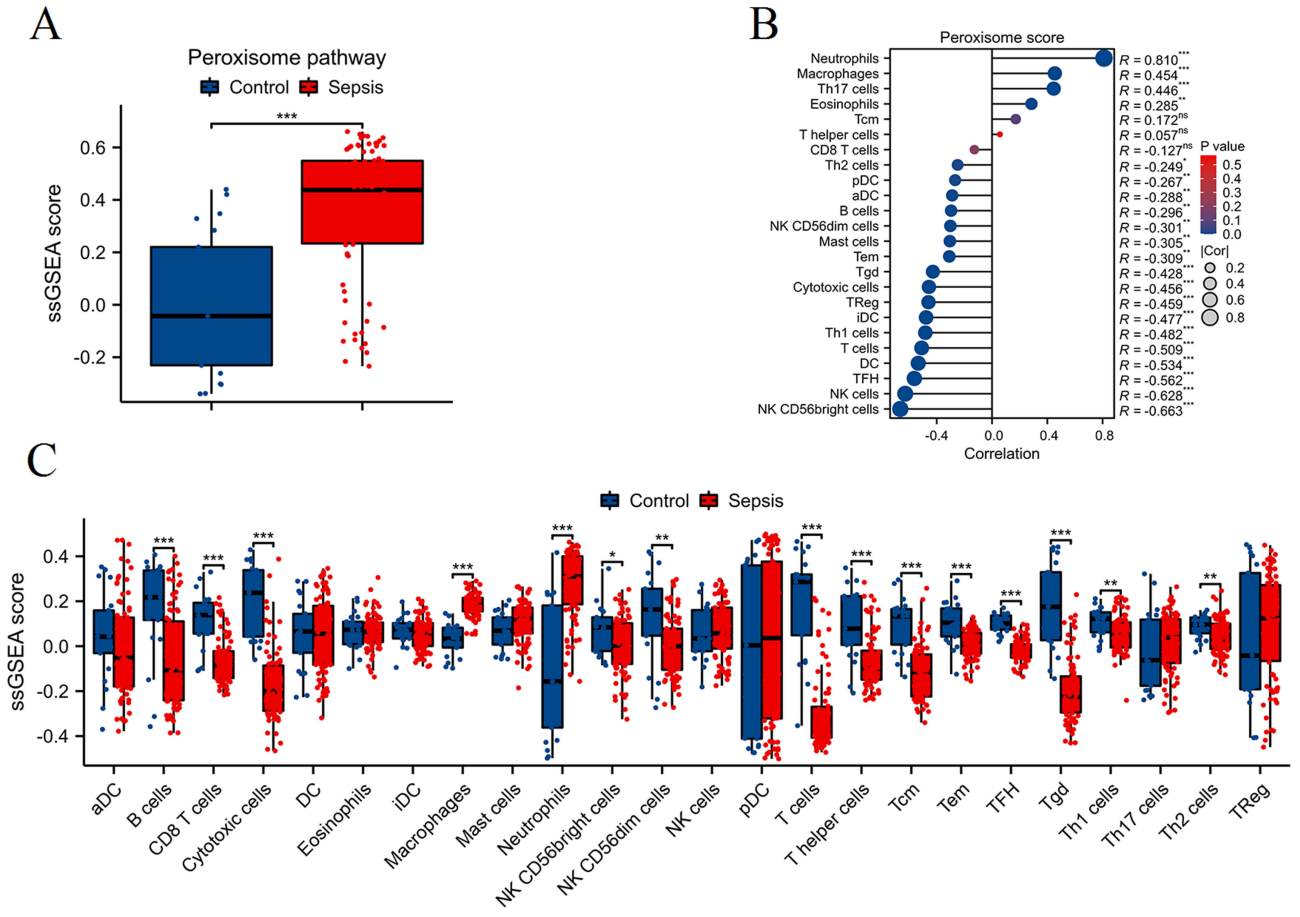

Figure 2 Evaluation of peroxisome pathway activity and its correlation with immune cells in sepsis. (**A**) Box plot comparing the ssGSEA score of the peroxisome pathway between control (blue, n = 21) and sepsis (red, n = 82) groups. Statistical significance was determined using the Wilcoxon rank-sum test (*** $p < 0.001$). (**B**) Correlation between peroxisome score and various immune cell types. The plot shows the Spearman correlation coefficients for each cell type, with significant values indicated (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns indicates no statistical difference). (**C**) Box plots comparing ssGSEA scores of various immune cell populations between control (blue, n = 21) and sepsis (red, n = 82) groups. Statistical significance was assessed using the Wilcoxon rank-sum test, and p-values were adjusted for multiple comparisons using the Benjamini-Hochberg FDR method (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

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immune cell infiltration were observed (Figure 2C). In the sepsis group, higher infiltration levels of cells such as neutrophils and macrophages were noted, while other cell types like NK CD56dim cells, B cells, T cells, and T helper cells showed decreased infiltration levels. In summary, peroxisome pathway activity is significantly elevated in sepsis and is associated with increased infiltration of specific immune cell types, notably neutrophils and macrophages, while being inversely correlated with NK cells. These findings suggest a potential interplay between peroxisome-related processes and immune cell dynamics in the context of sepsis.

Evaluation of Immune-Related Pathways and Their Correlation with Peroxisome Score

Figure 3A shows the comparison of ssGSEA scores for various immune-related pathways between control and sepsis groups. While pathways like antimicrobials, cytokines, cytokine receptors, interleukin receptors, and TNF family members showed significantly higher activity in sepsis ($p < 0.05$), indicating upregulated immune responses, the TCR signaling pathway was notably downregulated ($p < 0.001$). In order to explore the link between peroxisome pathway activity and immune response pathways, we performed a correlation analysis. As illustrated in Figures 3B–**E**, the scatter plots reveal the Spearman correlation between peroxisome scores and various immune pathways (BCR signaling pathway, interleukins receptor, natural killer cell cytotoxicity, and TNF family members). Notably, there are strong positive correlations between the activity of the peroxisome pathway and these immune pathways. The analysis indicates that immune-related pathways, especially those related to cytokines and TNF family members, are notably elevated in sepsis. Additionally, there is a strong positive correlation between peroxisome pathway activity and these immune pathways, highlighting a complex relationship between peroxisome functions and immune response in sepsis.

Assessment of Pathway Functions and Their Relationship with the Peroxisome Score

Figure 4A shows box plots comparing GSVA score of various pathways between control and sepsis groups. Numerous pathways, including KRAS signaling up, bile acid metabolism, IL2 STAT5 signaling, reactive oxygen species pathway, glycolysis, inflammatory response, apoptosis, PI3K-AKT-mTOR signaling, and TNFA signaling via NFKB, exhibited

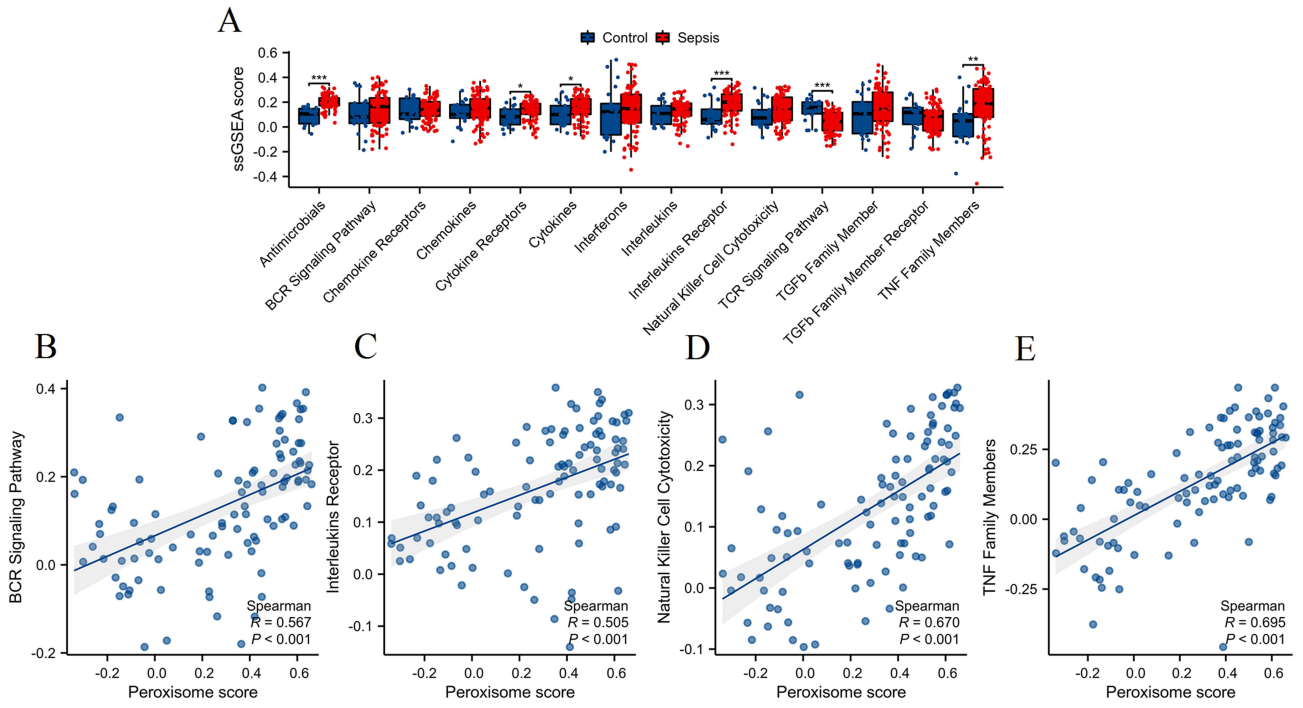

Figure 3 Evaluation of immune-related pathways and their correlation with peroxisome score in sepsis. (**A**) Box plot comparing ssGSEA score of various immune-related pathways between control (blue) and sepsis (red) groups. Statistical significance was assessed using the Wilcoxon rank-sum test, and p-values were adjusted for multiple comparisons using the Benjamini-Hochberg FDR method (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). (**B, C, D, E**) Scatter plots illustrating the Spearman correlation between peroxisome score and selected immune-related pathways.

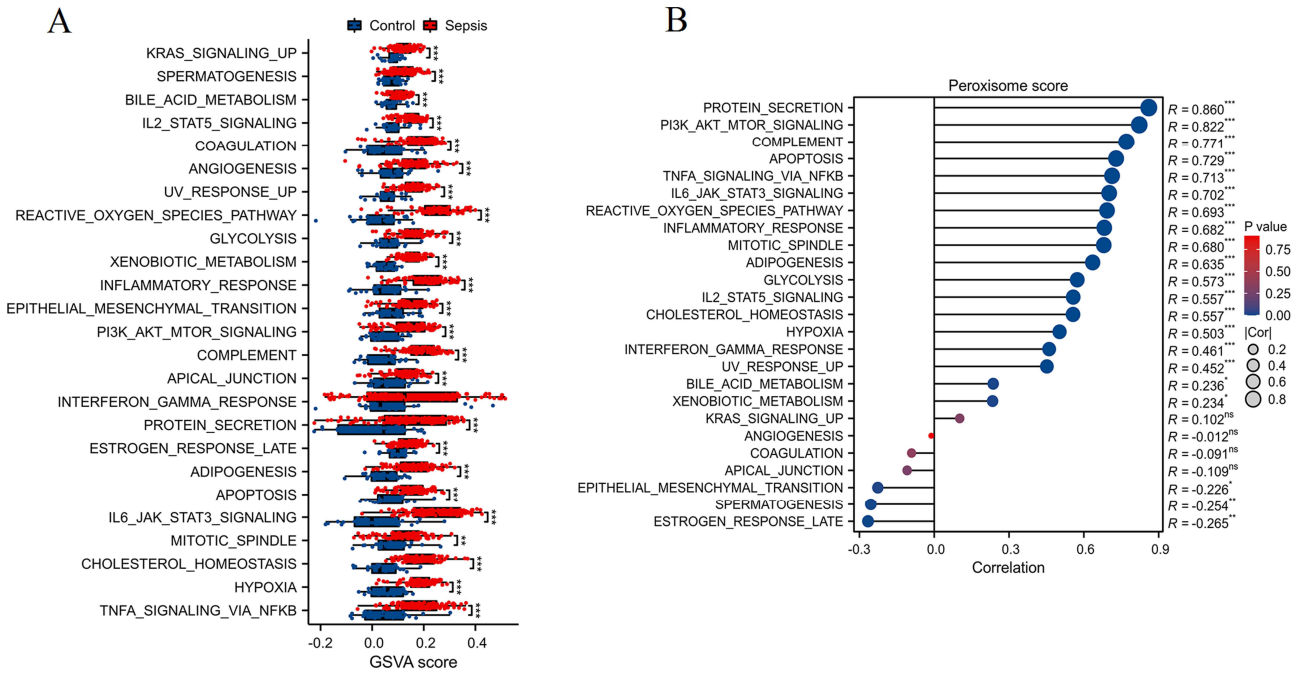

Figure 4 Evaluation of pathway activities and their correlation with peroxisome score. (**A**) Box plots comparing GSVA score of various pathways between control (blue) and sepsis (red) groups. Statistical significance was assessed using the Wilcoxon rank-sum test, and p-values were adjusted for multiple comparisons using the Benjamini-Hochberg FDR method (** $p < 0.01$, *** $p < 0.001$). (**B**) Correlation analysis between peroxisome score and differentially expressed pathways. The plot shows Spearman correlation coefficients for each pathway, with significant correlations indicated (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns indicates no statistical difference).

significantly higher activities in the sepsis group compared to the control group ($p < 0.01$ for significant differences). These findings indicate a pronounced upregulation of these pathways in pediatric sepsis. Figure 4B presents the correlation analysis between peroxisome score and the pathways that are differentially expressed. Spearman correlation coefficients (R) are used to evaluate the relationships. Notably, there are strong positive correlations between peroxisome score and pathways related to protein secretion, the PI3K-AKT-MTOR signaling pathway, complement activation, apoptosis, TNFA signaling via NFKB, IL6-JAK-STAT3 signaling, reactive oxygen species, and the inflammatory response. In summary, the GSVA analysis identified significant upregulation of multiple pathways in the sepsis group, highlighting immune and inflammatory responses as key features. Moreover, there is a considerable positive association between peroxisome activity and several critical pathways, notably those involved in protein secretion, signaling cascades, inflammation, and apoptosis, suggesting a potential functional interplay in the pathophysiology of sepsis.

Identification of Key Marker Genes for Sepsis Using Machine Learning Algorithms

Figure 5A depicts the LASSO regression results, indicating the optimal lambda value with the smallest binomial deviance. The LASSO model selected several genes, including ACAA1, ACSL1, ECI2, EPHX2, IDH1, ISOC1, PEX3, and PRDX5. Figure 5B shows the significance of genes identified by the Random Forest algorithm, ranked by their mean decrease accuracy. Higher values reflect greater importance, and the top 10 genes were selected. Logistic regression identified seven signature genes, as detailed in Table S3. Figure 5C shows a Venn diagram summarizing the overlapping genes identified by the three machine learning models. Both EPHX2 and IDH1 were consistently identified by all three algorithms, indicating their robust association with sepsis.

Construction and Validation of a Nomogram Based on EPHX2 and IDH1 Expression

Figure 6A illustrates the box plots that compare the expression levels of EPHX2 and IDH1 between the control and sepsis cohorts. In the GSE26378, the IDH1 gene demonstrated a markedly elevated expression in the sepsis cohort relative to the control cohort (*** $p < 0.001$), while EPHX2 exhibited a significantly reduced expression level in the

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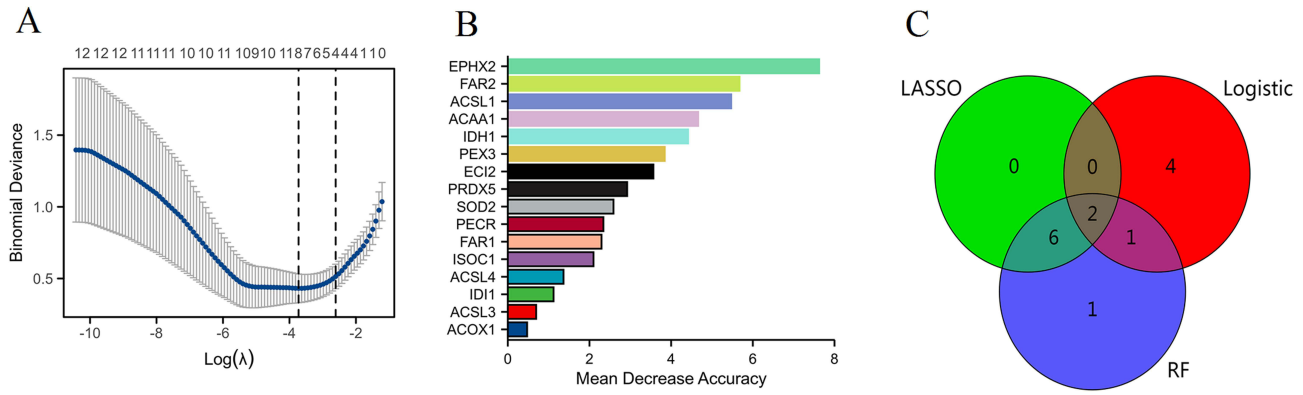

Figure 5 Identification of key marker genes for sepsis using machine learning algorithms. (**A**) LASSO regression analysis results showing the selection of lambda values. (**B**) RF analysis depicting the importance ranking of the genes based on the mean decrease accuracy. (**C**) Venn diagram illustrating the intersection marker genes identified by LASSO, RF, and Logistic Regression models.

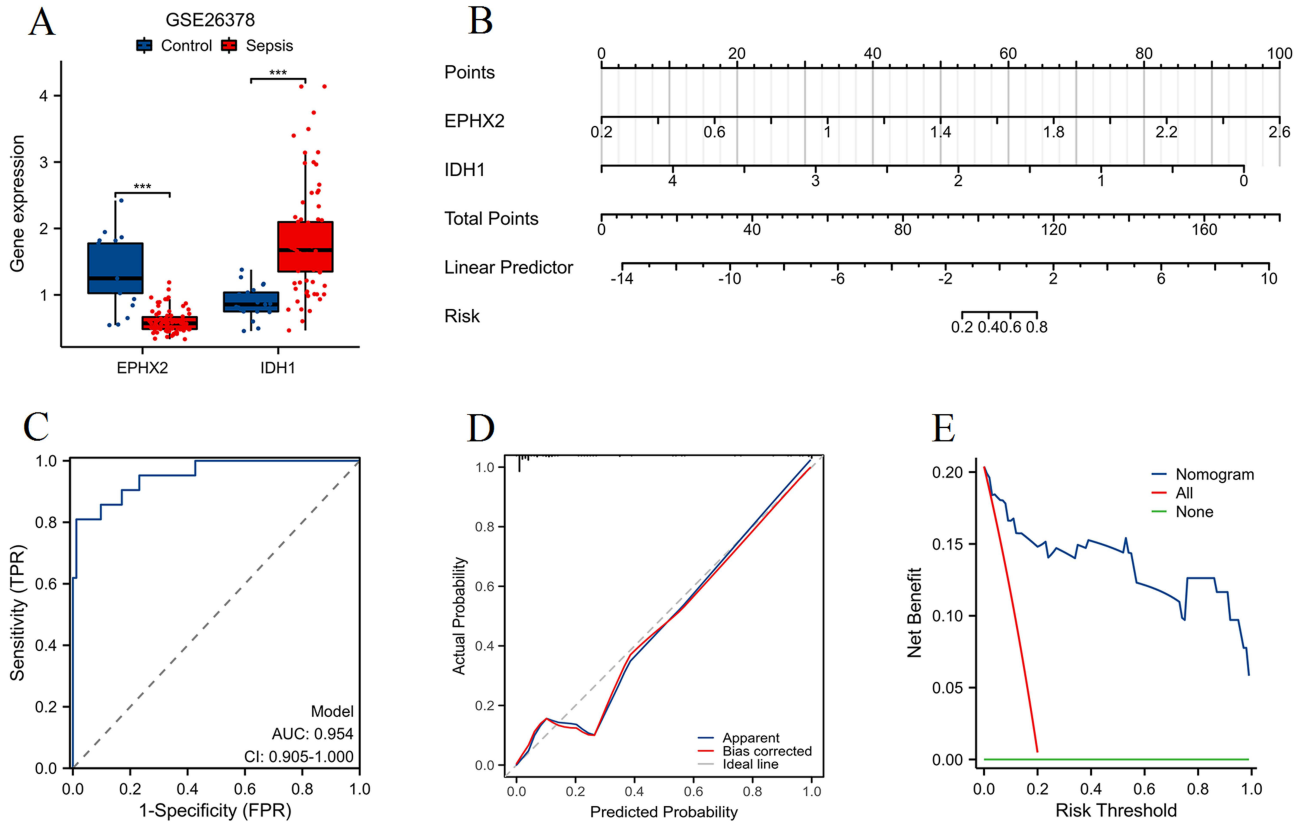

Figure 6 Construction of a nomogram based on EPHX2 and IDH1 expression. (**A**) Box plots comparing the expression levels of EPHX2 and IDH1 between control (blue) and sepsis (red) groups in the GSE26378 dataset. Statistical significance was determined using the Wilcoxon rank-sum test (*** $p < 0.001$). (**B**) Nomogram constructed to predict sepsis risk based on the expression levels of EPHX2 and IDH1. (**C**) ROC curve evaluating the discriminative ability of the nomogram. (**D**) Calibration plot comparing the predicted and actual probabilities of sepsis. (**E**) Decision curve analysis showing the net benefit of the nomogram model (blue line) across different risk thresholds, compared to treating all patients (red line) or none (green line) as having sepsis.

sepsis cohort (*** $p < 0.001$). The genes EPHX2 and IDH1 were chosen not only because they were consistently identified across LASSO, Random Forest, and Logistic Regression models, but also due to their high feature importance scores in these models. Moreover, the biological functions of EPHX2 and IDH1 are well-documented in the context of inflammation and oxidative stress, which are critical pathways in sepsis pathogenesis, ensuring both biological relevance and clinical interpretability. Figure 6B shows a nomogram designed to estimate the risk of sepsis based on EPHX2 and

IDH1 expression levels. Figure 6C displays the ROC curve, with an area under the curve (AUC) of 0.954, indicating high accuracy of the model. Figure 6D features the calibration plot, which reflects a strong correlation between predicted and actual sepsis probabilities, with the bias-corrected line closely aligning with the ideal line. Figure 6E presents the decision curve analysis, where the nomogram model (blue line) demonstrates a significant net benefit across various risk thresholds compared to the scenarios where all patients are assumed to have sepsis (red line) or none (green line). Furthermore, we conducted a re-evaluation of the nomogram's efficacy utilizing the GSE13904 dataset (Figures 7A–E) and GSE26440 (Figure S2). The findings align with those obtained from the GSE26378 dataset. In summary, the nomogram based on EPHX2 and IDH1 expression levels demonstrates robust predictive power and clinical utility for identifying sepsis patients.

Validation of EPHX2 and IDH1 in a Sepsis Animal Model

The expression levels of target genes EPHX2 and IDH1 were evaluated in LPS-induced sepsis and control groups (Figure 8). Compared to the control group, the relative mRNA level of EPHX2 was significantly decreased in the sepsis group (*p < 0.05). In contrast, the relative mRNA level of IDH1 was significantly increased in the sepsis group compared to the control group (**p < 0.01). These results suggest differential regulation of EPHX2 and IDH1 in response to LPS-induced sepsis.

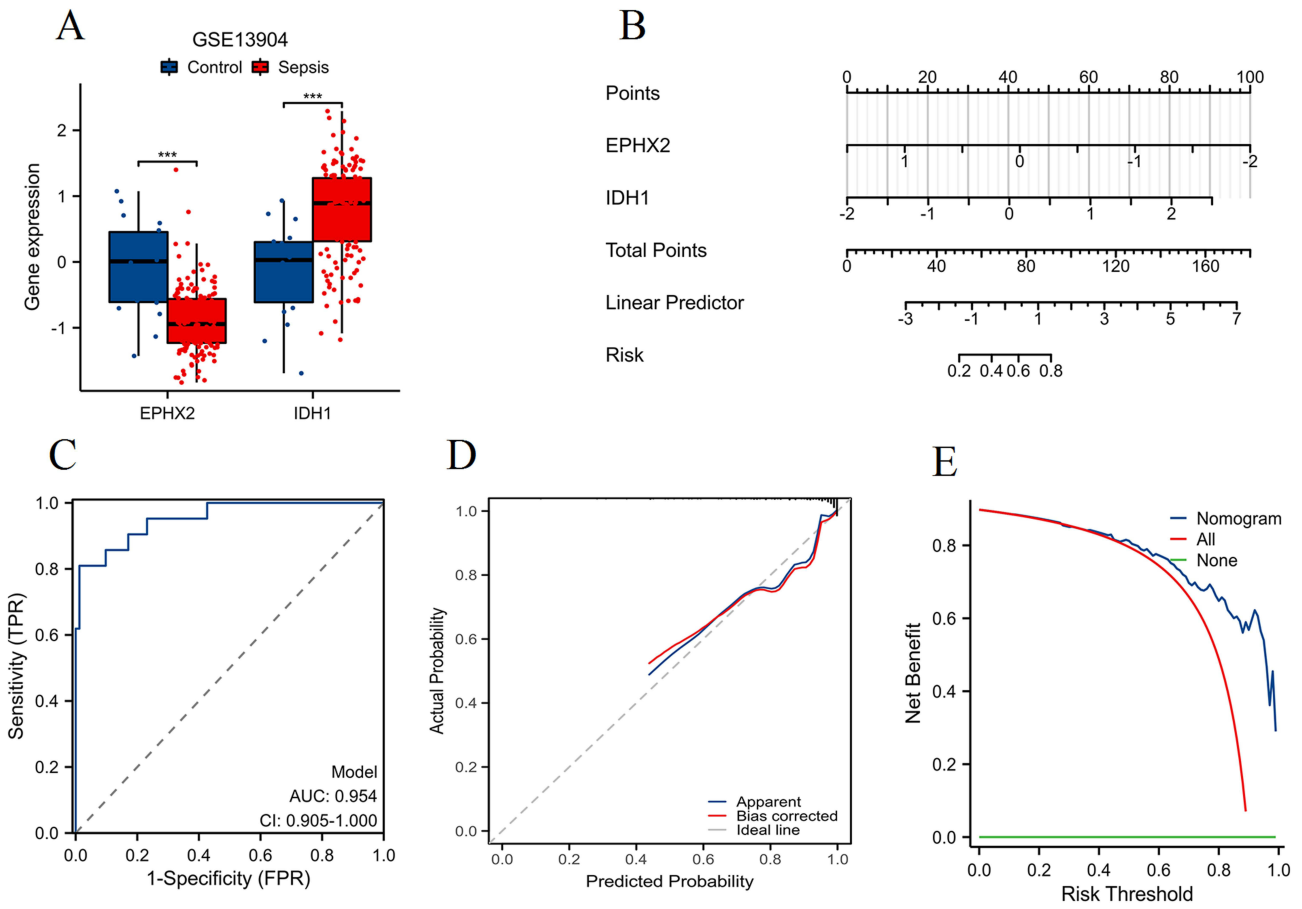

Figure 7 Validation of a nomogram by GSE13904 dataset. (**A**) Box plots comparing the expression levels of EPHX2 and IDH1 between control (blue) and sepsis (red) groups in the GSE13904 dataset. Statistical significance was determined using the Wilcoxon rank-sum test (*** p < 0.001). (**B**) Nomogram constructed to predict sepsis risk in patients based on the expression levels of EPHX2 and IDH1. (**C**) ROC curve evaluating the discriminative ability of the nomogram. (**D**) Calibration plot comparing the predicted and actual probabilities of sepsis. (**E**) Decision curve analysis showing the net benefit of the nomogram model (blue line) across different risk thresholds, compared to treating all patients (red line) or none (green line) as having sepsis.

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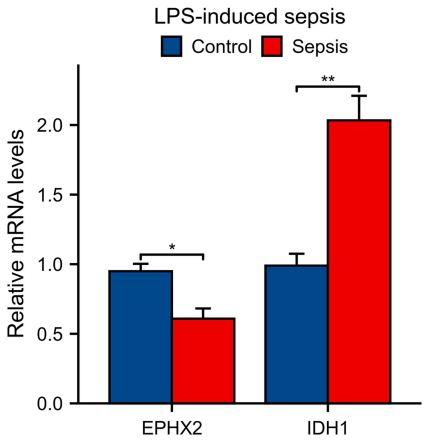

Figure 8 Validation of hub genes in a sepsis animal model. The bar graph shows the expression of EPHX2 and IDH1 in control (blue) and LPS-induced sepsis (red) groups. Statistical significance was determined using the Wilcoxon rank-sum test *p < 0.05, **p < 0.01.

Discussion

Peroxisomes are pivotal organelles involved in lipid metabolism, ROS detoxification, and the regulation of inflammatory responses. Their dysfunction has been implicated in various metabolic and inflammatory disorders, highlighting their potential role in the pathophysiology of complex diseases such as sepsis.[17–19] In sepsis, characterized by systemic inflammation and multi-organ failure, understanding the involvement of peroxisome-related processes could unveil novel diagnostic and therapeutic strategies.

Our study identified 16 DPGs in sepsis, indicating a significant alteration in peroxisome activity during the disease. The increased activity of the peroxisome pathway in septic patients underscores the organelle's potential involvement in modulating the sepsis-induced inflammatory response. This is consistent with previous reports suggesting that peroxisomes play a crucial role in controlling inflammation and oxidative stress.[20–22] Furthermore, the altered expression of these DPGs may offer novel therapeutic targets for sepsis management. By modulating peroxisome activity, it might be possible to enhance the resolution of inflammation and reduce tissue damage. However, it is important to note that bioinformatic predictions may not fully capture the functional dynamics at the protein level. Further validation using proteomics and other experimental methods is necessary to confirm these findings and to better understand the precise mechanisms at play. Future research should explore the mechanistic pathways through which these genes exert their effects, potentially leading to innovative interventions aimed at improving patient outcomes in sepsis.

The relationship between immune cell infiltration and the pathology of sepsis provides crucial insights into the disease's progression and severity.[23] Specifically, the correlations between peroxisome pathway activity and levels of immune cell infiltration elucidate the complex immune dynamics in sepsis. Positive correlations with neutrophils and macrophages, and negative correlations with NK cells, suggest a complex interaction between peroxisomes and the immune response. Neutrophils and macrophages are key players in the innate immune response, often associated with the release of pro-inflammatory cytokines and ROS, which can exacerbate tissue damage in sepsis.[24–27] The observed increase in peroxisome activity may reflect a compensatory mechanism to mitigate oxidative damage and modulate inflammatory responses, as supported by studies indicating peroxisomes' role in ROS detoxification, inflammation, and lipid metabolism.[28–31] Furthermore, our analysis revealed that pathways related to immunity, inflammation, and apoptosis are activated in sepsis, with a strong positive correlation between peroxisome activity and these pathways. This aligns with the concept that peroxisomes may influence immune responses and cell fate decisions during sepsis, potentially through the regulation of lipid mediators and ROS signaling.[32,33] The activation of apoptotic pathways in conjunction with increased peroxisome activity suggests a dual role of peroxisomes in both promoting and resolving inflammation, as part of the immune system's efforts to contain the infection and prevent excessive tissue damage.[22,34,35] However, our study did not account for potential sex differences (eg, using only male mice) or sepsis disease stages, which may limit the generalizability of our findings. Future work should incorporate both sexes and longitudinal sampling to capture stage-specific peroxisomal dynamics.

Our identification of key marker genes, EPHX2 and IDH1, further emphasizes the critical role of peroxisome-related processes in sepsis. Epoxide hydrolase 2 (EPHX2) is involved in the detoxification of epoxides and has been implicated in inflammatory processes.[36] Isocitrate dehydrogenase 1 (IDH1) plays a role in cellular metabolism and has been linked to oxidative stress responses.[37,38] The validation of these genes in an independent dataset strengthens their potential as biomarkers for sepsis and suggests that targeting these genes could modulate peroxisome function and inflammatory responses in septic patients. However, it is essential to consider potential confounders such as infection types, comorbidities, and treatment status, as these factors could influence gene expression and immune responses in patients. These confounders should be addressed in future studies to enhance the accuracy and relevance of the findings. The novelty of our study lies in the comprehensive analysis of peroxisome-related processes in sepsis, a relatively unexplored area. By integrating gene expression data with immune cell infiltration analyses and pathway activity assessments, we provide a holistic view of how peroxisomes may influence the immune dynamics in sepsis. Despite the limitations mentioned above, our findings not only enhance the understanding of peroxisome function in sepsis but also open new avenues for therapeutic interventions aimed at modulating peroxisome activity to improve clinical outcomes.

In conclusion, this study underscores the significant role of peroxisome-related processes in sepsis, particularly their interplay with immune cell dynamics and immune pathways. The identified marker genes, EPHX2 and IDH1, may serve as potential targets for future therapeutic strategies. Specifically, pharmacological modulation of EPHX2 or IDH1 could be explored to mitigate peroxisome-associated inflammation in sepsis. Additionally, these genes may have diagnostic utility as part of multi-marker panels for early sepsis detection or prognosis prediction. Further research is warranted to explore the mechanistic details of these interactions and to validate our findings in larger, independent cohorts.

Data Sharing Statement

The data utilized in this study were sourced from the GEO database, accessible at https://www.ncbi.nlm.nih.gov/geo/. The corresponding accession numbers are: GSE26378, GSE26440, and GSE13904.

Ethics Approval

The Animal Ethics Committee of Nanchang People's Hospital approved the animal experiment (Approval No. 20240161), which was conducted in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals. This study utilized publicly available datasets from the GEO database. Therefore, our study is exempt from approval based on national legislation guidelines, such as item 1 and 2 of Article 32 of the Measures for Ethical Review of Life Science and Medical Research Involving Human Subjects.

Disclosure

All authors declare no conflicts of interest in this work.

References

1. Eisenberg MA, Balamuth F. Pediatric sepsis screening in US hospitals. *Pediatr Res*. 2022;91:351–358.
2. Boomer LA, Feliz A. Sepsis management and the pediatric surgeon. *Seminar Pediatric Surg*. 2019;28:67–72.
3. Jacobi J. The pathophysiology of sepsis - 2021 update: part 2, organ dysfunction and assessment. *Amer J Health-System Pharm*. 2022;79 (6):424–436. doi:10.1093/ajhp/zxab393
4. Wiersinga WJ, van der Poll T. Immunopathophysiology of human sepsis. *EBioMedicine*. 2022;86:104363. doi:10.1016/j.ebiom.2022.104363
5. Okumoto K, Tamura S, Honsho M, Fujiki Y. Peroxisome: metabolic functions and biogenesis. *Advances in Experimental Medicine and Biology 1299*. 2020;3–17.
6. Mast FD, Rachubinski RA, Aitchison JD. Signaling dynamics and peroxisomes. *Current Opinion in Cell Biology*. 2015;35:131–136. doi:10.1016/j.ceb.2015.05.002
7. Fujiki Y. Peroxisome biogenesis and human peroxisome-deficiency disorders. *Procee Japan Acad Series B Phys Biolog Sci*. 2016; 92: 463–477.
8. Liu J, Zhou G, Wang X, Liu D. Metabolic reprogramming consequences of sepsis: adaptations and contradictions. *CMLS*. 2022;79(8):456. doi:10.1007/s00018-022-04490-0
9. Rimmelé T, Payen D, Cantaluppi V, et al. Immune cell phenotype and function in sepsis. *Shock*. 2016;45:282–291.
10. Amunugama K, Pike DP, Ford DA. The lipid biology of sepsis. *J Lipid Res*. 2021;62:100090. doi:10.1016/j.jlr.2021.100090
11. Kumar S, Saxena J, Srivastava VK, et al. The interplay of oxidative stress and ros scavenging: antioxidants as a therapeutic potential in sepsis. *Vaccines*. 2022;10(10):1575. doi:10.3390/vaccines10101575

12. Bavunoglu I, Genc H, Konukoglu D, et al. Oxidative stress parameters and inflammatory and immune mediators as markers of the severity of sepsis. *Journal of Infection in Developing Countries*. 2016;10(10):1045–1052. doi:10.3855/jidc.7585
13. Ayar G, Atmaca YM, Alışık M, Erel Ö. Effects of paraoxonase, arylesterase, ceruloplasmin, catalase, and myeloperoxidase activities on prognosis in pediatric patients with sepsis. *Clin Biochem*. 2017;50(7–8):414–417. doi:10.1016/j.clinbiochem.2016.12.013
14. Guerreiro MO, Petronilho F, Andrades M, et al. Plasma superoxide dismutase activity and mortality in septic patients [corrected]. *The Journal of Trauma*. 2010;69(6):E102–6. doi:10.1097/TA.0b013e3181dbb289
15. Dai W, Zheng P, Wu J, et al. Integrated analysis of single-cell RNA-seq and chipset data unravels PANoptosis-related genes in sepsis. *Front Immunol*. 2023;14:1247131. doi:10.3389/fimmu.2023.1247131
16. Bindea G, Mlecnik B, Tosolini M, et al. Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. *Immunity*. 2013;39(4):782–795. doi:10.1016/j.immuni.2013.10.003
17. Schrader M, Fahimi HD. Peroxisomes and oxidative stress. *Biochimica et biophysica acta 1763*. 2006;1763(12):1755–1766. doi:10.1016/j.bbamcr.2006.09.006
18. Yang XF, Shang DJ. The role of peroxisome proliferator-activated receptor γ in lipid metabolism and inflammation in atherosclerosis. *Cell Biology International*. 2023;47(9):1469–1487. doi:10.1002/cbin.12065
19. Sarkar C, Lipinski MM. Role and function of peroxisomes in neuroinflammation. *Cells*. 2024;13(19):1655. doi:10.3390/cells13191655
20. Terlecky SR, Terlecky LJ, Giordano CR. Peroxisomes, oxidative stress, and inflammation. *World J Biolog Chem*. 2012;3(5):93–97. doi:10.4331/wjbc.v3.i5.93
21. Nordgren M, Fransen M. Peroxisomal metabolism and oxidative stress. *Biochimie*. 2014;98:56–62. doi:10.1016/j.biochi.2013.07.026
22. Di Cara F, Andreoletti P, Trompier D, et al. Peroxisomes in immune response and inflammation. *Inter J Molecular Sci*. 2019;20(16):3877. doi:10.3390/ijms20163877
23. Nakamori Y, Park EJ, Shimaoka M. Immune deregulation in sepsis and septic shock: reversing immune paralysis by targeting PD-1/PD-L1 pathway. *Front Immunol*. 2020;11:624279. doi:10.3389/fimmu.2020.624279
24. Zhang H, Wang Y, Qu M, et al. Neutrophil, neutrophil extracellular traps and endothelial cell dysfunction in sepsis. *Clin Transl Med*. 2023;13(1):e1170. doi:10.1002/ctm2.1170
25. Ramoni D, Tirandi A, Montecucco F, Liberale L. Sepsis in elderly patients: the role of neutrophils in pathophysiology and therapy. *Int Emerg Med*. 2024;19(4):901–917. doi:10.1007/s11739-023-03515-1
26. Chen X, Liu Y, Gao Y, Shou S, Chai Y. The roles of macrophage polarization in the host immune response to sepsis. *Int Immunopharmacol*. 2021;96:107791. doi:10.1016/j.intimp.2021.107791
27. Wang Z, Wang Z. The role of macrophages polarization in sepsis-induced acute lung injury. *Front Immunol*. 2023;14:1209438. doi:10.3389/fimmu.2023.1209438
28. Schrader M, Costello JL, Godinho LF, Azadi AS, Islinger M. Proliferation and fission of peroxisomes - an update. *Biochimica et biophysica acta 1863*. 2016;1863(5):971–983. doi:10.1016/j.bbamcr.2015.09.024
29. Liu J, Lu W, Shi B, Klein S, Su X. Peroxisomal regulation of redox homeostasis and adipocyte metabolism. *Redox Biol*. 2019;24:101167. doi:10.1016/j.redox.2019.101167
30. Lodhi IJ, Semenkovich CF. Peroxisomes: a nexus for lipid metabolism and cellular signaling. *Cell Metab*. 2014;19(3):380–392. doi:10.1016/j.cmet.2014.01.002
31. Pawlak M, Lefebvre P, Staels B. Molecular mechanism of PPARα action and its impact on lipid metabolism, inflammation and fibrosis in non-alcoholic fatty liver disease. *J Hepatol*. 2015;62(3):720–733. doi:10.1016/j.jhep.2014.10.039
32. Jiang H, Nair V, Sun Y, Ding C. The diverse roles of peroxisomes in the interplay between viruses and mammalian cells. *Antiviral Research*. 2024;221:105780. doi:10.1016/j.antiviral.2023.105780
33. Pellegrino E, Aylan B, Bussi C, et al. Peroxisomal ROS control cytosolic mycobacterium tuberculosis replication in human macrophages. *J Cell Biol*. 2023;222(12). doi:10.1083/jcb.202303066
34. Di Cara F, Savary S, Kovacs WJ, Kim P, Rachubinski RA. The peroxisome: an up-and-coming organelle in immunometabolism. *Trends Cell Biol*. 2023;33(1):70–86. doi:10.1016/j.tcb.2022.06.001
35. Ferreira AR, Marques M, Ribeiro D. Peroxisomes and innate immunity: antiviral response and beyond. *Inter J Molecular Sci*. 2019;20(15):3795. doi:10.3390/ijms20153795
36. Li Y, Yu G, Yuan S, et al. Cigarette smoke-induced pulmonary inflammation and autophagy are attenuated in Ephx2-deficient mice. *Inflammation*. 2017;40(2):497–510. doi:10.1007/s10753-016-0495-z
37. Li S, Sun C, Gu Y, et al. Mutation of IDH1 aggravates the fatty acid-induced oxidative stress in HCT116 cells by affecting the mitochondrial respiratory chain. *Molecular Medicine Reports*. 2019;19(4):2509–2518. doi:10.3892/mmr.2019.9903
38. Grassian AR, Parker SJ, Davidson SM, et al. IDH1 mutations alter citric acid cycle metabolism and increase dependence on oxidative mitochondrial metabolism. *Cancer Res*. 2014;74(12):3317–3331. doi:10.1158/0008-5472.CAN-14-0772-T