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Peripheral blood transcriptome analysis in sepsis-associated acute kidney injury

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Abstract

Sepsis-associated acute kidney injury (S-AKI) presents significant clinical challenges, necessitating deeper insights into its molecular mechanisms. We conducted a comprehensive analysis—including Principal Component Analysis (PCA), differential gene expression, KEGG pathway enrichment, and Gene Set Enrichment Analysis (GSEA)—to elucidate the underlying pathways involved in S-AKI. PCA revealed distinct clustering between S-AKI patients and controls, with differential gene expression identifying 1,759 significant genes (912 upregulated, 847 downregulated). Notable upregulated genes included CYP19A1 and LY6G6F, while downregulated genes encompassed LRP1B and SIGLEC8. KEGG analysis highlighted pathways related to adaptive immune responses and infection, such as “Th1 and Th2 cell differentiation" and "Primary immunodeficiency.” GSEA pinpointed "Neutrophil extracellular trap formation" as the most significantly enriched pathway, underscoring neutrophil-mediated responses in S-AKI. Our findings suggest potential molecular targets for diagnosis and therapeutic intervention, providing a foundation for future research and improved clinical outcomes.

Keywords: Sepsis; Acute Kidney Injury; Transcriptome; Inflammation

1. Introduction

Sepsis is a life-threatening condition characterized by a dysregulated immune response to an infection[1]. A frequent and severe complication of sepsis is sepsis-associated acute kidney injury (S-AKI), where the kidneys are adversely affected due to the systemic inflammatory response. Approximately 60% of sepsis patients experience some degree of acute kidney injury, making it one of the most prevalent complications[2]. S-AKI is associated with a significantly higher risk of in-hospital mortality. Studies indicate that patients with S-AKI have an increased odds ratio for death compared to those with acute kidney injury (AKI) from other causes, with mortality rates reaching up to 50% in critically ill S-AKI patients [3].

Early diagnosis of S-AKI remains a formidable challenge due to factors related to the complexities of sepsis, limitations of current diagnostic criteria, and the intricate underlying pathophysiology[4]. To overcome these challenges, researchers are exploring novel biomarkers and advanced diagnostic techniques that may facilitate early detection of S-AKI. Emerging biomarkers such as Neutrophil Gelatinase-Associated Lipocalin (NGAL), Kidney Injury Molecule-1 (KIM-1), and Insulin-like Growth Factor Binding Protein 7 (IGFBP7) have shown promise in identifying early kidney injury[5] . However, these emerging biomarkers have certain limitations. Notably, the advent of RNA-sequencing technology presents a promising opportunity to identify biomarkers in the peripheral blood of S-AKI patients.

In this study, we employ RNA-sequencing of blood samples from S-AKI patients to identify significant gene signatures and unravel dysregulated pathways associated with this condition. Uncovering these gene signatures and dysregulated pathways could potentially lead to the development of novel biomarkers for early detection of S-AKI, facilitating timely

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intervention and improving clinical outcomes. Additionally, elucidating the underlying molecular mechanisms could pave the way for targeted therapeutic strategies, ultimately contributing to better management and treatment of this life-threatening complication.

2. Methods

2.1. Data Collection and Processing

The gene expression data for this study was obtained from the Gene Expression Omnibus (GEO) database, accession number GSE232404[6] . This dataset includes RNA-sequencing data from peripheral blood samples of S-AKI patients and healthy controls. The raw data files were downloaded and processed using appropriate bioinformatics pipelines for quality control, read alignment, and gene expression quantification.

2.2. Differential Gene Expression Analysis

To identify differentially expressed genes (DEGs) between the S-AKI and control groups, the limma package (Linear Models for Microarray and RNA-Seq Data) in R was employed. Limma implements an empirical Bayes method to moderate standard errors and improve the statistical power for identifying DEGs. Genes with an adjusted p-value (false discovery rate, FDR) less than 0.01 and an absolute log2 fold change greater than 1 were considered statistically significant and differentially expressed.

2.3. Functional Enrichment Analysis

The clusterprofiler package[7] in R was utilized to perform functional enrichment analysis of the identified DEGs. This package integrates gene annotation and statistical analysis methods to identify over-represented biological pathways and gene sets. Specifically, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment and Gene Set Enrichment Analysis (GSEA) were conducted to elucidate the dysregulated biological processes and pathways associated with S-AKI.

2.4. Visualization and Interpretation

The Gseavis R package was employed to visualize the GSEA results, providing intuitive representations of the enriched gene sets and their associations with the observed gene expression patterns.

3. Results

3.1. Principal Component Analysis and Differential Gene Expression

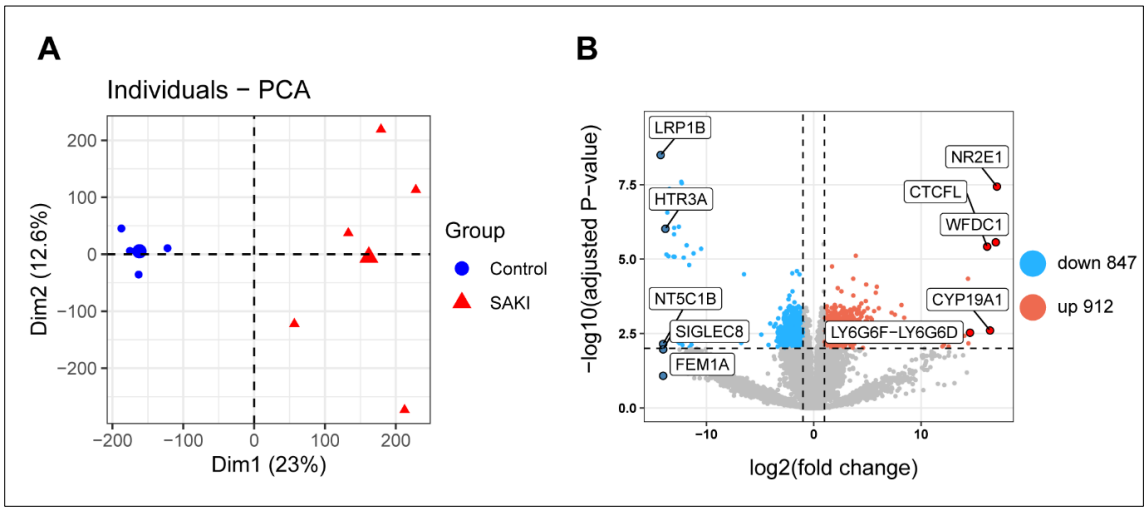

Figure 1 Analysis of Gene Expression in Control and SAKI Samples. (A) PCA plot showing the distribution of samples based on their gene expression profiles. (B) Volcano plot displaying differentially expressed genes between control and SAKI samples.

Principal Component Analysis (PCA) was performed to explore the variability and clustering of gene expression profiles among S-AKI patients and control samples. As shown in Figure 1A, PCA revealed a clear separation between the S-AKI samples and controls along principal component 1 (PC1), which accounts for 23% of the total variance. This indicates distinct gene expression patterns associated with S-AKI.

Differential gene expression analysis identified 1,759 significant genes, with 912 upregulated and 847 downregulated, based on an adjusted p-value < 0.01 and a log2 fold change threshold of ±1 (Figure 1B). Notable upregulated genes include CYP19A1, NR2E1, LY6G6F/LY6G6D, CTCFL, and WFDC1, while downregulated genes include LRP1B, HTR3A, NT5C1B, SIGLEC8, and FEM1A. These significant changes highlight potential biomarkers and provide insight into the molecular mechanisms underlying sepsis-associated acute kidney injury.

3.2. KEGG Pathway Enrichment Analysis

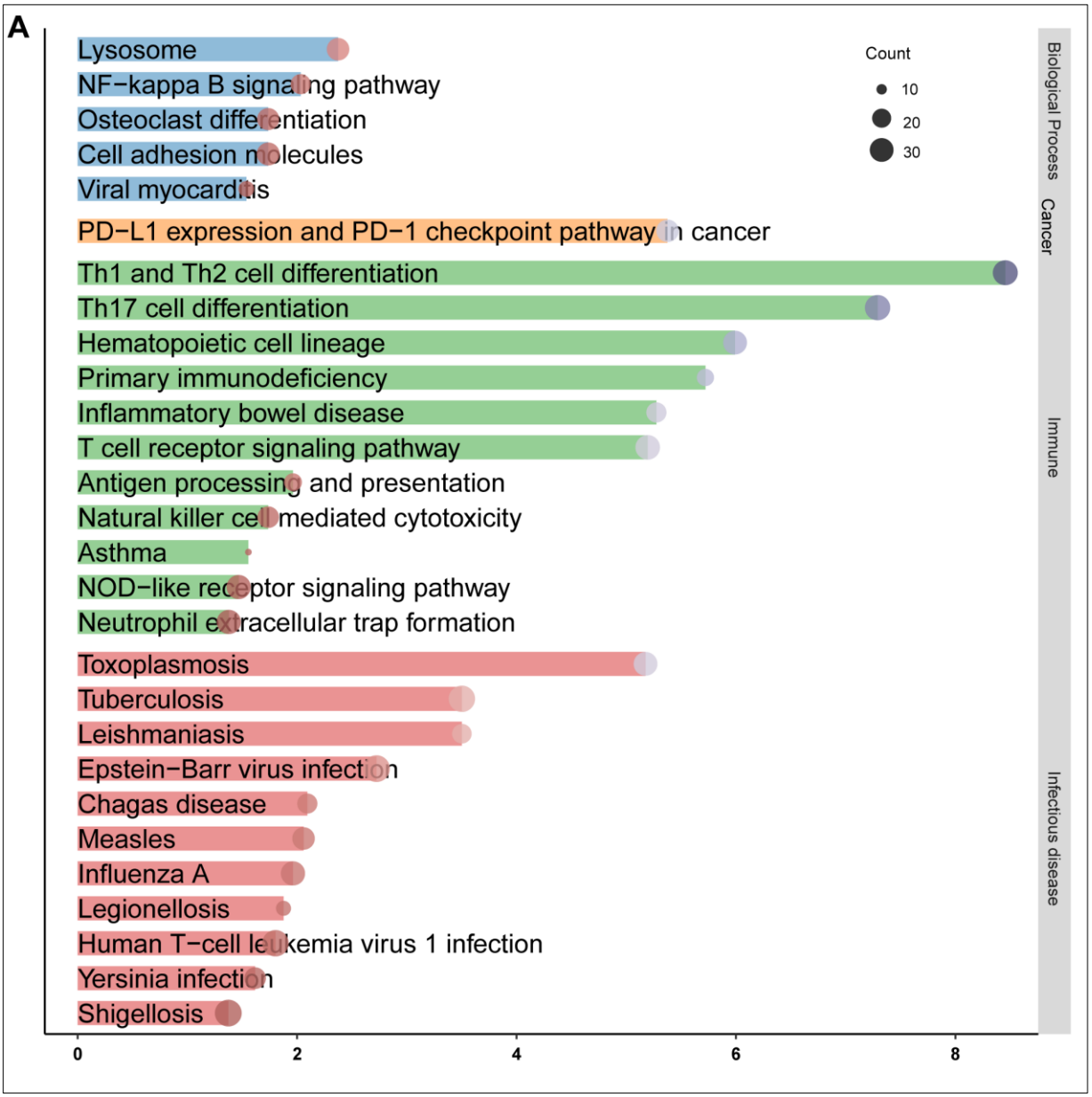

Figure 2 KEGG enrichment analysis. Bar plot summarizing the enriched biological processes, cancer pathways, immune responses, and infectious disease pathways identified among differentially expressed genes in SAKI samples.

KEGG pathway enrichment analysis was performed on the differentially expressed genes to elucidate the biological processes and pathways associated with S-AKI. The analysis revealed several significantly enriched pathways, providing insights into the molecular mechanisms underlying sepsis-associated acute kidney injury.

The top enriched pathways included "Th1 and Th2 cell differentiation" and "Th17 cell differentiation," suggesting a crucial role for adaptive immune responses in S-AKI. The "Hematopoietic cell lineage" pathway was also enriched, indicating potential alterations in blood cell development and function.

Notably, several pathways related to infectious diseases and immune responses were identified, including "Primary immunodeficiency," "PD-L1 expression and PD-1 checkpoint pathway in cancer," and "Inflammatory bowel disease." These findings highlight the complex interplay between infection, inflammation, and immune regulation in S-AKI.

The "T cell receptor signaling pathway" and "NF-kappa B signaling pathway" were among the enriched pathways, further emphasizing the involvement of both adaptive and innate immune responses. Additionally, pathways related to specific infections such as "Toxoplasmosis," "Tuberculosis," and "Epstein-Barr virus infection" were enriched, potentially reflecting the diverse range of infections that can lead to sepsis and subsequent kidney injury.

Other notable pathways included "Lysosome," "Antigen processing and presentation," and "Cell adhesion molecules," suggesting alterations in cellular processes and intercellular interactions. The enrichment of "Neutrophil extracellular trap formation" pathway underscores the potential role of neutrophil-mediated responses in S-AKI pathogenesis.

3.3. Gene Set Enrichment Analysis Results

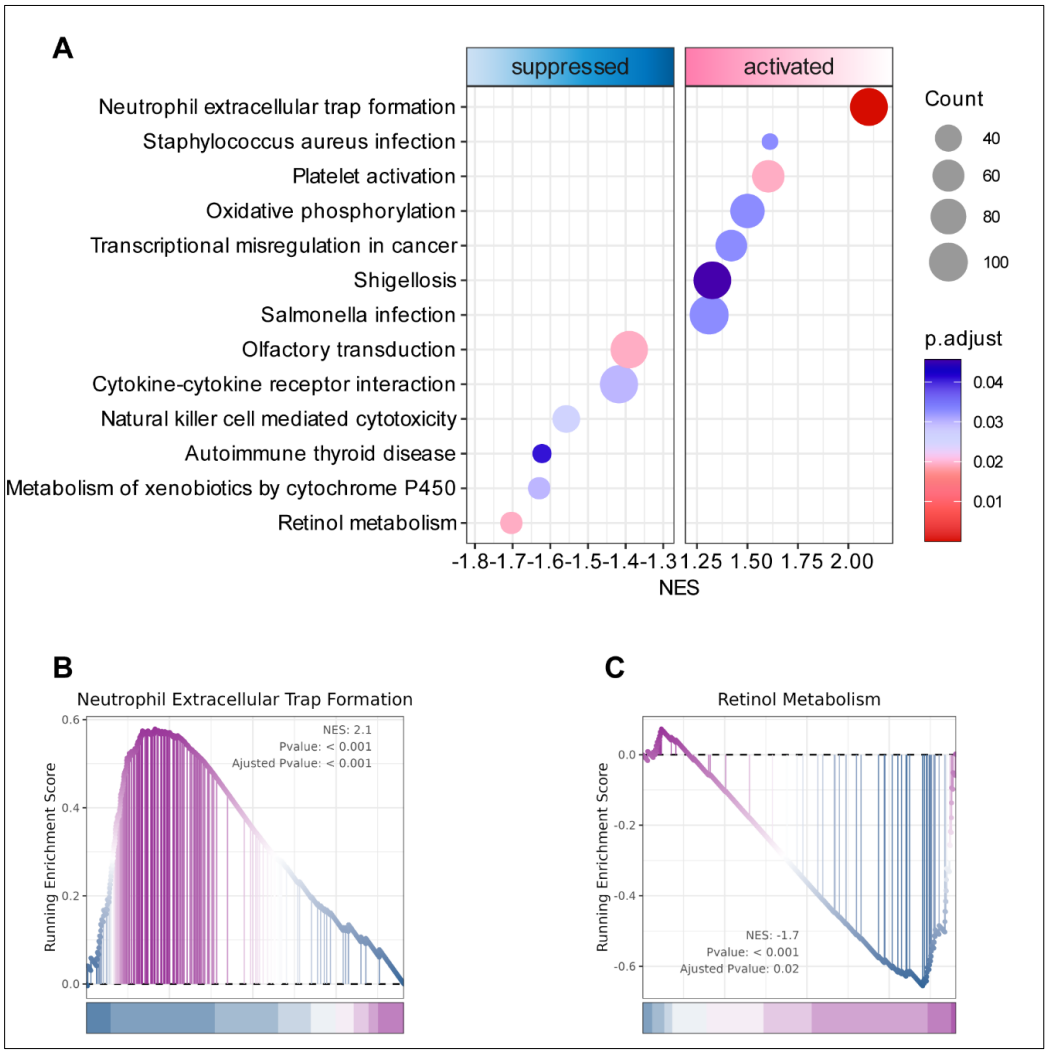

Figure 3 GSEA analysis. (A) Bubble plot depicting the pathway enrichment analysis. Pathways activated (red) or suppressed (blue) in SAKI samples are shown, with the x-axis representing the normalized enrichment score (NES). (B) GSEA plot for the “Neutrophil Extracellular Trap Formation” pathway, showing significant enrichment in SAKI samples. (C) GSEA plot for the “Retinol Metabolism” pathway, showing significant suppression in SAKI samples.

GSEA was performed to identify significantly enriched gene sets associated with S-AKI, providing insight into the biological processes and pathways that are altered in this condition. The most significantly enriched pathway was "Neutrophil extracellular trap formation" (NES = 2.1), indicating a strong involvement of neutrophil-mediated immune responses in S-AKI. This aligns with the known role of neutrophils in sepsis and acute kidney injury (Figure 3A). Similarly, several pathways related to infection and immune responses were positively enriched, including "Staphylococcus aureus infection" (NES = 1.61), "Salmonella infection" (NES = 1.31), and "Shigellosis" (NES = 1.33). Additionally, pathways such as "Platelet activation" (NES = 1.60) and "Oxidative phosphorylation" (NES = 1.50) showed significant positive enrichment.

Conversely, several pathways exhibited significant negative enrichment, including "Retinol metabolism" (NES = -1.70), "Metabolism of xenobiotics by cytochrome P450" (NES = -1.63), and "Natural killer cell mediated cytotoxicity" (NES = -1.56) (Figure 3A). Detailed enrichment plots further illustrate these findings; the "Neutrophil extracellular trap formation" pathway was markedly activated (Figure 3B), while "Retinol metabolism" was the most suppressed pathway (Figure 3C). These results highlight significant alterations in various biological processes, suggesting potential therapeutic targets and providing deeper insights into the mechanisms underlying S-AKI.

4. Discussion

In this study, we explored the underlying molecular mechanisms of S-AKI through a series of comprehensive analyses, including PCA, differential gene expression, KEGG pathway enrichment, and GSEA. Our findings provide significant insights into the distinct gene expression patterns and biological processes associated with S-AKI, thereby contributing to the existing body of knowledge in this field.

Our PCA revealed a clear segregation between S-AKI patients and control samples, reinforcing the notion that specific gene expression profiles are intimately linked with S-AKI. This distinct separation is consistent with previous studies that identified unique transcriptional signatures associated with sepsis and organ dysfunction[8,9].

Differential gene expression analysis identified 1,759 significant genes, with 912 upregulated and 847 downregulated. Notably, upregulated genes such as WFDC1, was reported to be an important prognotstic gene in sepsis[10], while LY6G6F was reported to regulate immune in many ways[11].

The KEGG enrichment analysis emphasized key pathways such as "Th1 and Th2 cell differentiation" and "Th17 cell differentiation," highlighting the importance of adaptive immune responses in S-AKI. This is congruent with previous studies that delineated the role of Th1/Th2 imbalance and Th17-mediated inflammation in sepsis pathogenesis[12]. Similarly, enriched pathways such as "Primary immunodeficiency" and "PD-L1 expression and PD-1 checkpoint pathway in cancer" underscore the potential for immunotherapies in S-AKI management[13], reflecting the recent interest in the modulation of immune checkpoints to alleviate septic conditions[14].

GSEA underscored the activation of the "Neutrophil extracellular trap (NET) formation" pathway (NES = 2.1), emphasizing a pivotal role of neutrophil-mediated responses in S-AKI. This corroborates with literature indicating the dual role of NETs in sepsis; while they can trap and kill pathogens, excessive NET formation can cause additional tissue damage, exacerbating organ dysfunction[15,16]. Targeting NETs as a therapeutic intervention has shown promise in experimental models, opening potential clinical applications for managing S-AKI.

Conversely, pathways such as "Retinol metabolism" (NES = -1.70) and "Metabolism of xenobiotics by cytochrome P450" (NES = -1.63) were significantly suppressed. The downregulation of these pathways indicates disrupted metabolic processes and detoxification mechanisms, aligning with known metabolic impairments in sepsis[17]. Previous studies have demonstrated that vitamin A (retinol) and cytochrome P450 enzymes play critical roles in maintaining epithelial integrity and detoxifying harmful substances, deficits of which are implicated in the pathology of S-AKI[17,18].

In summary, our comprehensive analysis identifies key genes and pathways involved in S-AKI, highlighting significant immune and metabolic disruptions, and suggests potential molecular targets for diagnosis and therapeutic intervention, thereby paving the way for future research and clinical approaches to improve patient outcomes.

While our study provides valuable insights into the molecular mechanisms of S-AKI, it has several limitations. Firstly, the sample size is relatively small, which may limit the generalizability of our findings. Secondly, our analysis relies primarily on transcriptomic data without validation at the protein level, which is crucial for confirming the functional

relevance of the identified genes and pathways. Lastly, the study's cross-sectional design captures a single time point and may not fully represent the dynamic changes occurring during the progression of S-AKI.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare no conflict of interest.

Author contributions

Visualization: Xuehuan Wen; Writing – original draft: Xuehuan Wen, Zhengshuang Liu; Writing – review & editing: Xuehuan Wen, Zhengshuang Liu.

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References

[1] Singer, M. *et al.* The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *Jama* 315, 801-810, doi:10.1001/jama.2016.0287 (2016).

[2] He, F. F. *et al.* Sepsis-induced AKI: From pathogenesis to therapeutic approaches. *Front Pharmacol* 13, 981578, doi:10.3389/fphar.2022.981578 (2022).

[3] Zarbock, A., Gomez, H. & Kellum, J. A. Sepsis-induced acute kidney injury revisited: pathophysiology, prevention and future therapies. *Curr Opin Crit Care* 20, 588-595, doi:10.1097/mcc.0000000000000153 (2014).

[4] Peerapornratana, S., Manrique-Caballero, C. L., Gómez, H. & Kellum, J. A. Acute kidney injury from sepsis: current concepts, epidemiology, pathophysiology, prevention and treatment. *Kidney Int* 96, 1083-1099, doi:10.1016/j.kint.2019.05.026 (2019).

[5] Wei, W., Zhao, Y., Zhang, Y., Shou, S. & Jin, H. The early diagnosis and pathogenic mechanisms of sepsis-related acute kidney injury. 18, doi:doi:10.1515/biol-2022-0700 (2023).

[6] Ma, S. R., Ma, Q., Ma, Y. N. & Zhou, W. J. Comprehensive analysis of ceRNA network composed of circRNA, miRNA, and mRNA in septic acute kidney injury patients based on RNA-seq. *Front Genet* 14, 1209042, doi:10.3389/fgene.2023.1209042 (2023).

[7] Wu, T. *et al.* clusterProfiler 4.0: A universal enrichment tool for interpreting omics data. *Innovation (Cambridge (Mass.))* 2, 100141, doi:10.1016/j.xinn.2021.100141 (2021).

[8] Antcliffe, D. B. *et al.* Transcriptomic Signatures in Sepsis and a Differential Response to Steroids. From the VANISH Randomized Trial. *Am J Respir Crit Care Med* 199, 980-986, doi:10.1164/rccm.201807-1419OC (2019).

[9] Mohammed, A., Cui, Y., Mas, V. R. & Kamaleswaran, R. Differential gene expression analysis reveals novel genes and pathways in pediatric septic shock patients. *Sci Rep* 9, 11270, doi:10.1038/s41598-019-47703-6 (2019).

[10] He, H. *et al.* Identification of a novel sepsis prognosis model and analysis of possible drug application prospects: Based on scRNA-seq and RNA-seq data. *Front Immunol* 13, 888891, doi:10.3389/fimmu.2022.888891 (2022).

[11] Wang, B. & Liu, H. Identification of potential immune/diagnosis related gene-immunocyte subtype networks in extracellular immune response to respiratory syncytial virus infection. *Virus research* 321, 198906, doi:10.1016/j.virusres.2022.198906 (2022).

[12] Martin, M. D., Badovinac, V. P. & Griffith, T. S. CD4 T Cell Responses and the Sepsis-Induced Immunoparalysis State. *Front Immunol* 11, 1364, doi:10.3389/fimmu.2020.01364 (2020).

[13] Wiersinga, W. J. & van der Poll, T. Immunopathophysiology of human sepsis. *EBioMedicine* 86, 104363, doi:10.1016/j.ebiom.2022.104363 (2022).

[14] Nakamori, Y., Park, E. J. & Shimaoka, M. Immune Deregulation in Sepsis and Septic Shock: Reversing Immune Paralysis by Targeting PD-1/PD-L1 Pathway. *Front Immunol* 11, 624279, doi:10.3389/fimmu.2020.624279 (2020).

[15] Zhang, H. *et al.* Neutrophil extracellular traps mediate m(6)A modification and regulates sepsis-associated acute lung injury by activating ferroptosis in alveolar epithelial cells. *International journal of biological sciences* 18, 3337-3357, doi:10.7150/ijbs.69141 (2022).

[16] Zhang, H. *et al.* Neutrophil, neutrophil extracellular traps and endothelial cell dysfunction in sepsis. *Clinical and translational medicine* 13, e1170, doi:10.1002/ctm2.1170 (2023).

[17] Lewis, A. J., Billiar, T. R. & Rosengart, M. R. Biology and Metabolism of Sepsis: Innate Immunity, Bioenergetics, and Autophagy. *Surgical infections* 17, 286-293, doi:10.1089/sur.2015.262 (2016).

[18] Dixon, J., Lane, K., Macphee, I. & Philips, B. Xenobiotic metabolism: the effect of acute kidney injury on non-renal drug clearance and hepatic drug metabolism. *Int J Mol Sci* 15, 2538-2553, doi:10.3390/ijms15022538 (2014).