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Original Article

Transcriptomic Identification of Key Genes in Human Peripheral Blood Mononuclear Cells for the Prognosis of Sepsis

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Abstract

Background and objectives: To find key genes related to the prognosis of sepsis through transcriptomic sequencing of peripheral blood mononuclear cells (PBMCs) from survival and death septic patients.

Methods: 78 septic patients were recruited in the emergency intensive care unit of Xijing Hospital from Apr. 1, 2018, to Jun. 30, 2020, and divided into the survival (n = 67) and death (n = 11) groups. Their PBMCs were collected for transcriptomic sequencing. The differentially expressed genes (DEGs) were identified by bioinformatic analyses and validated by RT-PCR.

Results: Bioinformatic analyses revealed 457 DEGs. The GO function and KEGG Pathway analyses suggested that the ENO1 and AK4 were potential target genes; RT-PCR results exhibited that ENO1, but not AK4, gene expression significantly decreased in the death group compared to the survival.

Conclusions: ENO1 may be a potential prognostic biomarker for sepsis in humans.

Introduction

Sepsis is a common clinical disease in an emergency department, with high morbidity and mortality worldwide. The global incidence of sepsis is over 19 million people annually, and around 14 million of them survive and be discharged from the hospital with various prognoses. Among them, one-half of patients recover, one-third die within two years, and one-sixth suffer from other severe persistent injuries.[1] The dysregulated host response to infections is a hallmark of sepsis, which may cause multiple organ inflammatory damages. Early detection of sepsis, prompt administration of antibiotics, fluid therapy, and appropriate treatment with vasopressors are essential to reduce sepsis-induced organ damage and mortality.[2] Studies indicated that septic patients may have different outcomes even though they receive the same treatments.[3] Therefore, explorations of specific factors modulating sepsis prognosis and clinically individualized therapeutic strategy are of great significance for the improvement of sepsis treatment.

To date, serum procalcitonin and C-reactive protein levels remain the two major biomarkers for the diagnosis and prognosis of sepsis, but they still have limitations in distinguishing sepsis from some other inflammatory states.[4] Besides, studies have revealed that interleukin (IL)-27 has a diagnostic value for both child and adult patients with sepsis.[5,6] Soluble triggering receptor expressed

Keywords: Transcriptomic sequencing; Prognosis of sepsis; ENO1.

Abbreviations: DEG, differentially expressed genes; ENO1, Enolase-1; GO, gene ontology; IL, interleukin; KEGG, Kyoto Encyclopedia of Genes and Genomes; PBMC, peripheral blood mononuclear cells; TREM-1, triggering receptor expressed on myeloid cells-1.

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on myeloid cells-1 (TREM-1) has been studied to predict the severity of bacterial infection that can also be used for the diagnosis of sepsis.[7] Moreover, both presepsin and neutrophil CD64 are regarded as potential biomarkers for the diagnosis and prognosis of sepsis.[8–10] Through a long-term observation and evaluation of multiple biomarkers for the diagnosis and prognosis of sepsis, there is not a single biomarker reliable in clinics.[11] Hence, continual exploration of potential efficient biomarkers for the diagnosis and prognosis of sepsis will be of significance.

In the current study, we conducted transcriptomic sequencing of peripheral blood mononuclear cells (PBMCs) from the septic patients admitted to our hospital with different outcomes to explore potential key regulatory genes and significant biomarkers for the prognosis of sepsis. Our study may provide more evidence and reference for the clinical management of septic patients.

Materials and methods

Patients

The data of septic patients in the emergency intensive care unit of Xijing Hospital of Air Force Medical University from Apr. 1, 2018, to Jun. 30, 2020, were obtained for the study. Inclusion criteria were 1) age >18 years old; 2) familiar with the study and willing to participate; 3) disease course less than 24 hours; 4) diagnostic criteria was "Sepsis 3.0": scores of patients with suspected sepsis = infection factor + rapid sequential organ Failure assessment ≥2 (i.e., contained 2 of the following 3 items: definite abnormal changes in consciousness, respiratory rate >22 breaths/min, systolic blood pressure less than 100 mmHg) and 5) without antibiotic treatment after onset. The exclusion criteria were that the inspection indicators and scores did not meet the evaluation criteria. The study was approved by the Ethics Committee of the Xijing Hospital (approval number KY20193106, Supplementary File 1).

Grouping and PBMC transcriptomic sequencing

A total of 78 patients were diagnosed with sepsis and were divided into the survival (67 cases) and death (11 cases) groups according to their outcomes. The peripheral blood samples from 13 patients that survived and 3 from patients that died were collected within 24 hours after admission. PBMCs were isolated using a Lymphocyte Separation kit for Human Peripheral Blood (Dakewe Biotech, Shenzhen, China). Total RNAs from individual PBMC samples were extracted using TRIGene Reagent (Cat. No. P118-05, Genstar, Beijing, China), according to the manufacturer's instructions. The extracted RNAs were subjected to transcriptomic sequencing by OmicShare (Guangzhou, China) in Illumina HiSeqTM.

Differentially expressed gene screening

After filtering the off-machine data to obtain high-quality data, the Cufflinks program was used to calculate the relative expression value of each gene to obtain new transcripts using a false discovery rate of <0.01 to ensure the data reliability, combined with a requirement of a protein unique peptide ≥1. The obtained genes were then subjected to expression analysis and statistics. The differentially expressed genes (DEGs) were identified using the criteria of Fold Change >1.5 and $P < 0.05$. Subsequently, the potential functions and pathways of these DEGs were analyzed using the Gene Ontology (GO) and Kyoto encyclopedia of Genes and Genomes (KEGG) Pathway databases. The top 20 GO terms and KEGG pathways with the smallest Q values were collected.

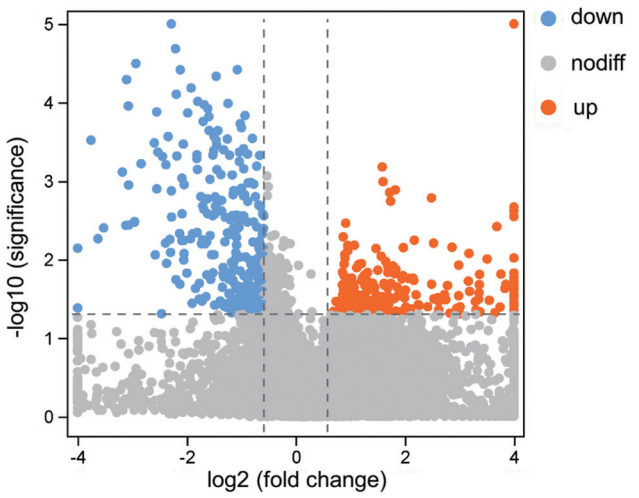


Fig. 1. Volcano plot of DEGs between the two groups (Survival vs. Death). The red dots represent up-regulated genes, the blue dots represent down-regulated genes, and the gray dots represent that there is no significant difference in the genes between the two groups.

Quantitative real-time PCR verification

The primers for specific genes were synthesized by Beijing Zhongke Yutong Biological. Total RNAs of PBMCs from the survival (67 patients) and death (11 patients) groups were extracted and reversely transcribed into cDNAs using a reverse transcription kit (TaKaRa, Japan). PCR reaction was performed using the following reagents of 0.1 mL fluorescent quantitative PCR 8-strip tubes (Shanghai Sangon Biotechnology): 1 μL of cDNA product, 0.5 μL of forwarding primer, 0.5 μL of reverse primer, 5 μL of SYBR® Green (TaKaRa, Japan), and 3 μL of RNase-free ionized water. The reaction program was set as follows: 95°C for 2 min, 40 cycles of 95°C for 20 s, and 58°C for 15 s. The $2^{-\Delta\Delta Ct}$ method was used to measure the CT values of each group and their relative expression levels.

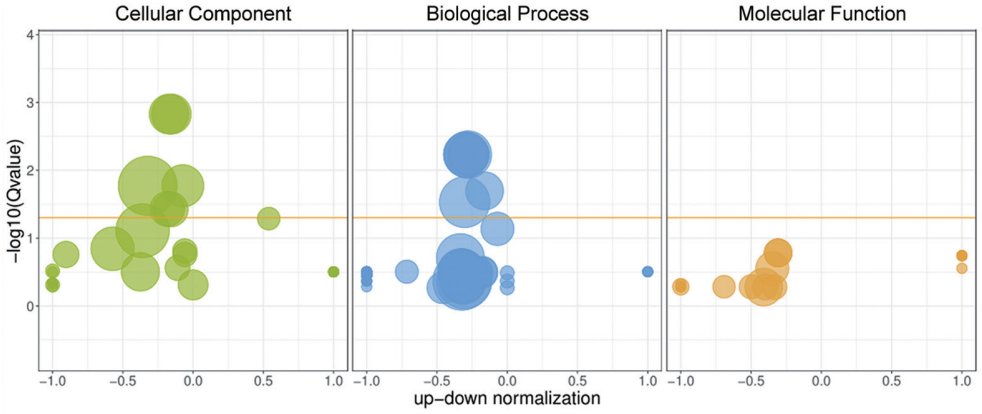
Statistical analysis

The relative expression levels of the sequenced genes were statistically analyzed using edgeR software. All data were expressed as mean ± standard deviation and analyzed by GraphPad Prism 8.0 software. Comparison of data between two groups was performed by unpaired t-test. A *p*-value of <0.05 was considered statistically significant.

Results

The DEGs between the survival and death groups

A total of 475 DEGs were identified between these two groups. Compared with the survival group, 209 genes were up-regulated and 266 were down-regulated in the death group (Fig. 1).



ID	Descrption
GO:0006810	transport
GO:0051234	establishment of localization
GO:0051179	localization
GO:0006950	response to stress
GO:0002376	immune system process
GO:0050896	response to stimulus
GO:0016192	vesicle−mediated transport
GO:0044421	extracellular region part
GO:0005615	extracellular space
GO:0005737	cytoplasm
GO:0005576	extracellular region
GO:0031410	cytoplasmic vesicle
GO:0031982	vesicle
GO:0031012	extracellular matrix
GO:0044444	cytoplasmic part
GO:0005829	cytosol
GO:0005764	lysosome
GO:0005768	endosome
GO:0030234	enzyme regulator activity
GO:0098772	molecular function regulator

Fig. 2. GO term classification of DEGs. Left: statistical bubble chart of GO term classification. The bubble size represents the specific number of genes in this secondary category in the GO database. The orange line represents the threshold value of Q value = 0.05. Different color represents different ontologies: green-cellular component, blue-biological process, and orange-molecular function. Right: top 20 enriched GO terms of DEGs.

GO functional annotation analysis of DEGs

GO functional annotation analysis of DEGs found that the top 20 GO terms with the most significant enrichment were mainly involved in the biological processes of transport, responses to stress, and also immune response, as well as cellular component of the extracellular region part (Fig. 2).

KEGG pathway enrichment analysis of DEGs

Meanwhile, the KEGG pathway enrichment analysis predicted that metabolism-related pathways were dominant, and these genes were mainly involved in the metabolism of various substances in the body: purine metabolism, glycolysis, sulfur metabolism, sphingolipid metabolism, biosynthesis of antibiotics, microbial metabolism, selenocompound metabolism, and vitamin B6 metabolism (Fig. 3).

Targeted gene identification

After GO function and KEGG enrichment analysis, the genes with the most obvious differences in the metabolism-related pathways were identified, including PAPSS2, ENO1, FDPS, AK4, PAPSS1, GALM, RPIA, and BPGM. Their relative expression was further analyzed by a heat map, and the relative expression levels of ENO1 and AK4 between these groups were different, with ENO1 significantly up-regulated and AK4 significantly down-regulated in the survival group (Fig. 4).

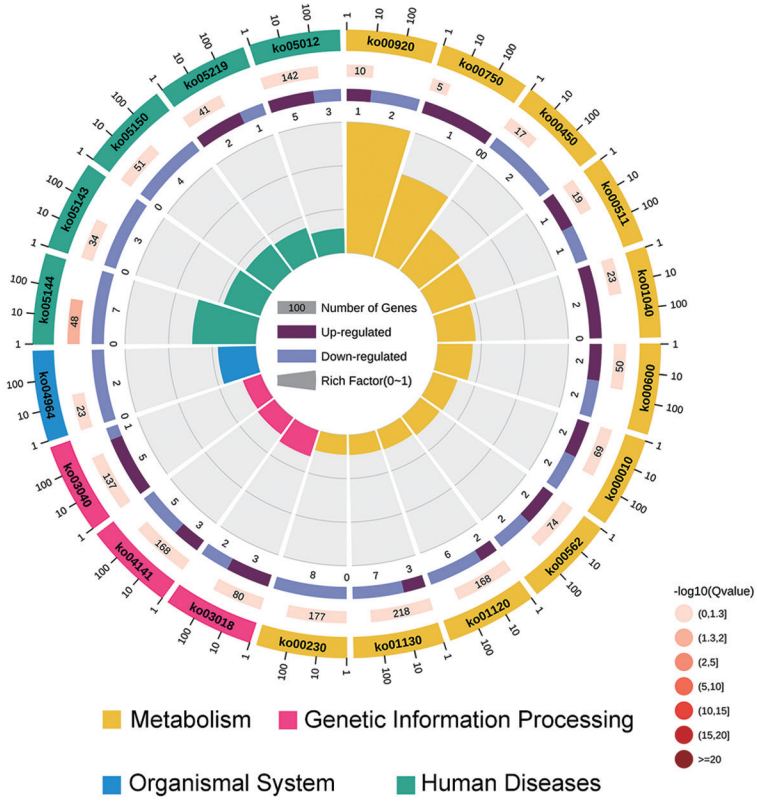
Expression of targeted genes in patients

ENO1 and AK4 were then selected as the targeted genes related to sepsis. To verify their expression profiles, the relative levels of their gene transcripts in PBMCs from these two groups of patients were quantified by RT-qPCR (Fig. 5). The results revealed that compared with the survival group, the levels of AK4 mRNA transcripts in the death group had no significant change, but ENO1 was down-regulated significantly ($p < 0.05$). The decrease in the levels of ENO1 expression may be related to the poor prognosis of septic patients.

Discussion

Sepsis prognosis-related biomarkers are crucial for the management of septic patients. To date, most researches focus on the pathogenesis of sepsis, but there are few investigations on the sepsis prognosis and its related underlying mechanisms and determinants. Recent studies lie in the analysis of three biomarkers of inflammation (interleukin-6, procalcitonin, and C-reactive protein)[12] and the SOFA, SAPS II, and APACHE II scores, which are correlated with the prognosis of sepsis, but there is yet clear conclusion. Despite these, the levels of serum IL-27, TREM-1, presepsin expression, and several CD64+ neutrophils have also been reported to be potential biomarkers for the diagnosis, prognosis, and also treatment of sepsis.[13] Although the clinical value and verification of those biomarkers have not been well-evaluated, there are still increasing biomarkers studied in sepsis-related research to expand the cohort.[14] Identification of novel key biomarkers will increase the possibility of sepsis therapeutic strategy and provide more perspective for predicting the prognosis of sepsis.

The gene expression analysis has played a certain guiding role in the clinical diagnosis, treatment, and related scientific research of sepsis.[15] Due to the difficulty to obtain tissue samples directly from the source of infection, whole blood and PBMCs have become the subjects of most gene expression studies of sepsis.[16] In this study, we performed PBMC sequencing and performed bioin-



ID	Descrption
ko00920	Sulfur metabolism
ko00600	Sphingolipid metabolism
ko01130	Biosynthesis of antibiotics
ko01120	Microbial metabolism in diverse environments
ko00450	Selenocompound metabolism
ko00230	Purine metabolism
ko00511	Other glycan degradation
ko00010	Glycolysis / Gluconeogenesis
ko01040	Biosynthesis of unsaturated fatty acids
ko00562	Inositol phosphate metabolism
ko00750	Vitamin B6 metabolism
ko05144	Malaria
ko05012	Parkinson disease
ko05150	Staphylococcus aureus infection
ko05143	African trypanosomiasis
ko05219	Bladder cancer
ko04964	Proximal tubule bicarbonate reclamation
ko03018	RNA degradation
ko04141	Protein processing in endoplasmic reticulum
ko03040	Spliceosome

Fig. 3. KEGG pathway enrichment of DEGs. Left: Pie chart of KEGG pathway enrichment. The outer yellow circle represents metabolism-related genes, red represents genetically information processing genes, blue represents organ system-related genes, and the green represents human disease-related genes. The pink inner circle means the degree of difference: the darker the color, the more significant the difference. The inner purple circle represents the number of up-regulated genes in the KEGG, and blue represents the number of down-regulated genes. Right: top 20 enriched KEGG pathways of DEGs

formatics analysis to find the key genes with obvious differences and important functions between the survival and death of patients with sepsis. The results revealed that 209 DEGs were up-regulated and 266 were significantly down-regulated between these two groups. Further, GO function and KEGG enrichment analyses predicted that the DEGs were mainly involved in the metabolic pathways. The abnormal metabolism in sepsis is a hot research topic in recent years. Studies have found that lactate produced by aerobic glycolysis during the process of sepsis inhibits the activation of immune cells, thereby aggravating the infection.[17] Moreover, glycolytic metabolism is critical for sepsis-induced septic cardiomyopathy.[18] Hence, abnormal metabolic function is indeed a key factor involved in the development and prognosis of sepsis.[19]

The glycolysis pathway is also crucial for the inflammatory responses of the innate immune system. A previous study has reported that lactate acts as a main mediator to promote macrophage M2 polarization mainly through HIF-1α-dependent signaling in bone marrow-derived macrophages.[20] Meanwhile, lactate induces preferential differentiation of monocytes into M2 macrophages through metabolic reprogramming in a dose-dependent manner.[21] Enolase-1 (ENO1) is a crucial glycolytic enzyme expressed in the cytoplasm, nucleus, and membrane of eukaryotic cells. It mainly catalyzes the dehydration of 2-phosphoglycerate into phosphoenolpyruvate during the catabolic glycolysis pathway.[22] Through bioinformatics screening and clinical sample comparison, we found that ENO1 was significantly reduced in the death group, and the down-regulated ENO1 gene expression may be related to the poor prognosis of sepsis. ENO1 is a key regulator of the protein-protein interaction and the activation of signaling pathways during glycolysis,[23] the invasion and metastasis of tumors,[24] and the malignant phenotype of cancer stem cells.[25] It has been identified as an important target for tumor-targeted therapy.[26] However, ENO1-related research was rare in sepsis. ENO1 intervenes and regulates cellular energy metabolism through the cellular Warburg effect,[27] and affects immune cell activities by enhancing monocyte invasion ability.[28] These functions are correlated with the prognosis of sepsis, so further studies of the ENO1-related mechanisms regulating the prognosis of sepsis can be taken into consideration to reveal key factors for the poor prognosis of sepsis. Some limitations still existed in this study, for example, the sample size was relatively small and the sample homogeneity was not well. We will further expand the sample size in our subsequent study.

Future perspective

Sepsis, as a common clinical disease in an emergency department,

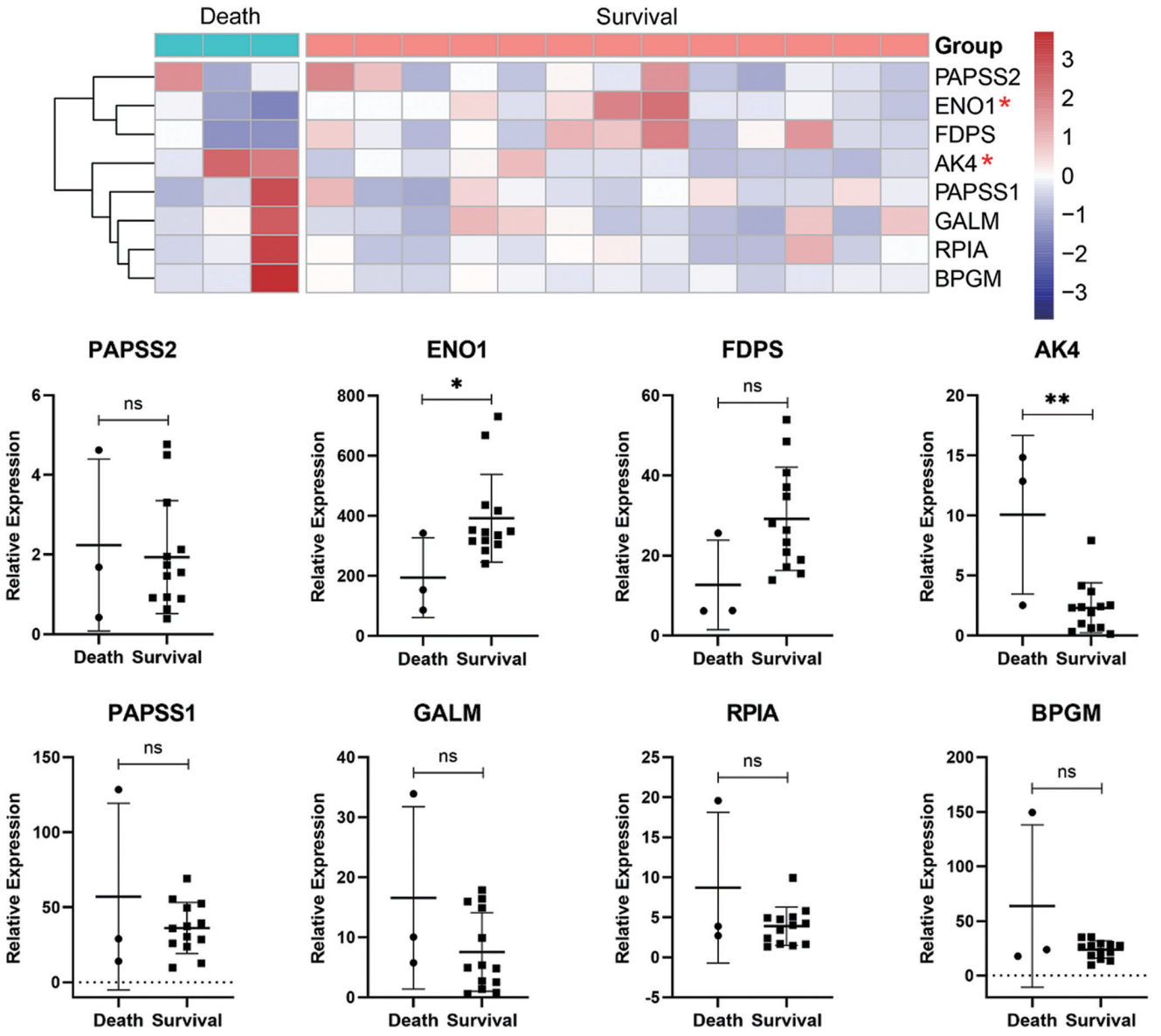


Fig. 4. Expression heatmap between differential genomes. Up: Blue is down-regulated, and red is up-regulated. The darker the color, the more obvious the difference. Down: Relative expression levels of these genes from sequencing data were quantified. *$p < 0.05$, **$p < 0.01$.

still has a high mortality rate. The application of biomarkers has important clinical significance for the diagnosis, prognosis, and treatment of sepsis. Although increasing biomarkers are being identified and studied in recent years, the majority of them have not been studied in the clinic validation and application. Therefore, clinical evaluation of these sepsis biomarkers requires more time and effort to explore.

Conclusions

In conclusion, we sequenced PBMCs from septic patients and identified that the ENO1 gene expression was significantly down-regulated in the death group. This suggests that low ENO1 expression may be related to the poor prognosis of sepsis. Therefore, our findings may provide a reference direction for the prognosis of sepsis.

Supporting information

Supplementary material for this article is available at https://doi.org/10.14218/JERP.2022.00023.

Supplementary File 1. STROBE Checklist.

Acknowledgments

None.

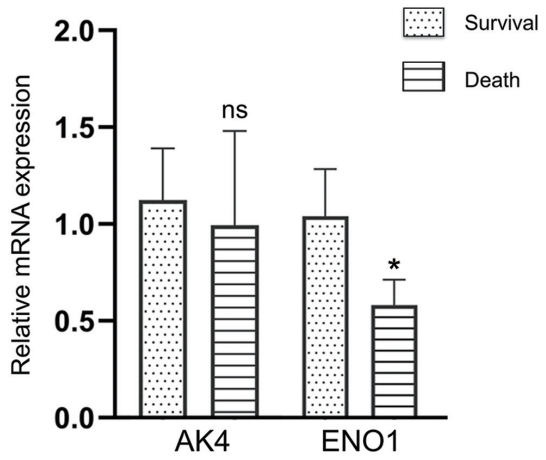


Fig. 5. Relative expression levels of ENO1 and AK4 from PBMC samples between the survival and death groups. ns means no statistical significance compared to the survival; *$p < 0.05$versus the survival group.

Funding

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Conflict of interest

The authors have no conflicts of interest related to this publication.

Author contributions

JGX and XCW proposed the review aim. YX, YJW, and JGX collected and analyzed data. YX performed the experiments. YX and JGX drafted the manuscript. XCW supervised the whole work and revised the manuscript. All authors have read and approved the manuscript.

Ethical statement

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in studies involving human participants were in accordance with the ethical standards of the Ethics Committee of the Xijing Hospital (approval number KY20193106) and with the Declaration of Helsinki (as revised in 2013). Written informed consent was obtained from the patient.

Data sharing statement

Technical appendix, statistical code, and dataset available from the corresponding author at william@fmmu.edu.cn.

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