Peer Review File

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Reviewer A

Comment 1: In the introduction of the manuscript, it is necessary to clearly indicate the characteristics of infiltrating immune cells in sepsis and the therapeutic significance.
Reply 1: We have already described the relevant content in the manuscript. (The pathogenic mechanism of the development of sepsis is immune dysfunction, including early immune system overactivation and late immune suppression. In the early stage of sepsis, immune cells such as macrophages secrete a large number of pro-inflammatory factors and chemokines, which aggravate the inflammatory reaction. In the late stage of sepsis, immune cell inactivation and endotoxin tolerance mediated by changes in immune cell phenotype, decreased antigen presentation and increased release of anti-inflammatory factors cause immunosuppression, which makes the host susceptible to secondary infection and increases mortality.)
Changes in the text: None.

Comment 2: All figures are not clear enough. It is recommended to provide clearer figures again.
Reply: The figures have been replaced with the original high-definition version and the compression during image production has been reduced. Thank you for your suggestion.
Changes in the text: Figure 1-9.

Comment 3: It is recommended to increase the functional study of key genes.
Reply 1: We have already described the relevant content in the manuscript. (We performed GO and KEGG pathway analysis to identify the potential biological functions of the 80 DEARGs, and a total of 564 biological processes (BPs), 60 cellular components (CC), 41 molecular functions (MFs), and 55 KEGG signaling pathways were obtained. We used the R language package “ggplot2” to visualize the results, and subsequently identified the top 10 enriched GO terms and the top 30 enriched KEGG signaling pathways (Figure 2A,2B). The results showed that the most significantly enriched GO ms involved macroautophagy, autophagy regulation, and autophagosome organization.)
Changes in the text: None.

Comment 4: The characteristics of sepsis microenvironment and the progress of treatment research should be added to the discussion.
Reply: We have already described the characteristics of sepsis microenvironment (Multiple immune cells play an essential role in sepsis: neutrophils migrate to the site of infection to exert phagocytosis and bactericidal action; dendritic cells are the most functionally proficient antigen-presenting cells (APCs) which can efficiently take up, process, and present antigens; T cells participate in adaptive immune response; and NK cells perform nonspecific direct killing of the pathogen.) and the progress of treatment research in the manuscript(A previous study

indicated that autophagy could prevent monocytes from undergoing apoptosis and promote their differentiation into macrophages. Induction of autophagy can alleviate the damage of excessive inflammatory injury by inducing the transition of macrophages from the M1 phenotype to the M2 phenotype. Moreover, some immune cells, including CD8 T cells, naïve CD4 T cells, activated CD4 memory T cells, and resting NK cells, have been reported to be negatively correlated with hub ARGs. In contrast to our findings, one study found that autophagy deficiency in T cells was associated with increased apoptosis of CD4+ and CD8+.).
Changes in the text: None.

Comment 5: What is the progress of autophagy in the development and treatment of sepsis? It is recommended to include relevant content.
Reply: We have already described the relevant content in the manuscript. (Autophagy is a key host defense mechanism against pathogens, plays a vital part in the induction and regulation of innate immune cell inflammatory responses, and influences the development of sepsis. Autophagy may play a protective role in sepsis through the direct clearance of pathogens, the neutralization of microbial toxins, the modulation of cytokine release, and the promotion of antigen presentation. In addition, activation of autophagy in sepsis patients can induce the formation of neutrophil extracellular traps (NETs), thereby alleviating damage to host organs. Apoptosis of CD4+ T cells is an important cause for the suppression of immune function in the pathogenesis of sepsis. Increased expression of the autophagy-negative regulator Mitofusin 2 (Mfn2) suppresses immune function during sepsis by inhibiting autophagy through increasing the apoptosis of CD4+ T cells. Thus, autophagy plays an important role in immune regulation in patients with sepsis.)
Changes in the text: None

Comment 6: The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as “Bioinformatics analysis based on immune-autophagy-related lncRNAs combined with immune infiltration in bladder cancer, Transl Androl Urol, PMID: 34532269”. It is recommended to quote this article.
Reply: We have modified our text as advised.
Changes in the text: We have modified our text as advised (see Page 4, line 110)

Comment 7: How to provide candidate targets for the treatment of sepsis based on the results of
this study? It is recommended to include relevant descriptions in the discussion.
Reply: The high expression of ARGs may cause some immune cells to activate in the early stage of sepsis, leading to the immune-activated microenvironment. Regulating the expression of ARGs may become a treatment for organ function damage caused by cytokine storm in early stage of sepsis, which may help guide immune modulators to achieve immune homeostasis.
Changes in the text: We have modified our text as advised (see Page 14, line 446-451)

Comment 8: This study is based on bioinformatics analysis. It is recommended to increase in vivo and in vitro experimental studies, which may be more meaningful.
Reply: Indeed, experimental verification of genes is the best way to prove the results of research.

However, the number of our clinical samples is not enough to carry out experimental verification. In order to remedy this defect, we used other data sets for verification to achieve the same effect as far as possible.
Changes in the text: None.

Reviewer B

Comment 1: First, the title needs to indicate the research design of a bioinformatics analysis.
Reply 1: We have modified our text as advised.
Changes in the text: Title.

Comment 2: Second, the abstract needs some revisions. The background did not indicate the clinical needs for identifying the biomarkers and what the knowledge gap is on this research focus. The methods need to describe the clinical sample in the dataset, the diagnosis of sepsis, the training and validation datasets, and how the ceRNA network was established. The conclusion needs more detailed comments for the clinical implications of the findings.
Reply 2: We have modified our text as advised. The criteria of sepsis have been added to the method. All AUC values in the training and validation samples have been added to the results. More comments for the clinical implications of the findings have been added to the conclusion.
Changes in the text: We have modified our text as advised (see Page 4, line 131-132, Page 3, line 84-88, Page 15, line 478-480).

Comment 3: Third, the introduction of the main text needs to review known biomarkers associated with sepsis, analyze their knowledge gaps and limitations, and explain why the focus on the molecular diagnostic markers related to autophagy is more important. The authors need to further explain why they emphasized “diagnostic”, not “prognostic”, since the diagnosis of sepsis should not be a major clinical concern.
Reply 1: We have modified our text as advised. Sepsis sometimes exhibits a high inflammatory response pattern, followed by an immunosuppressive period during which multiple organ dysfunction may occur. Therefore, biomarkers that can reflect the immune status of sepsis patients may be a new way to predict, identify, or provide new methods for treating sepsis. The significance of diagnosis includes the judgment of different stages of a disease
Changes in the text: We have modified our text as advised (see Page 3, line 84-88).

Comment 4: Fourth, in the methodology of the main text, please describe the datasets used in detail including the clinical factors, diagnosis, and prognosis outcomes. The AUC≥0.70 is unsatisfactory I suggest the authors to report the sensitivity and specificity values of the biomarkers. In statistics, please ensure $P<0.05$ is two-sided.
Reply 1: We have modified our text as advised. The criteria of sepsis have been added to the method. In our study, the AUC values of hub ARGs were all above 0.85 in test dataset and 0.95 in validation datasets, indicating that the 7 hub ARGs had diagnostic values with excellent specificity and sensitivity and were determined to be diagnostic markers. In statistics, $P<0.05$ is two-sided
Changes in the text: We have modified our text as advised (see Page 4, line 131-132).

Reviewer C

Comment 1: The authors need to describe the two populations used to generate GSE28750 and GSE95233 particularly the clinical status of the septic patients and the corresponding control groups. GSE28750 did not use control subjects but post-operative subjects who were not septic.
Reply 1: GSE28750 did use the samples of hospital staff with no known concurrent illnesses (n=20) as Healthy controls. GSE28750 includes post-operative subject's data, but our study did not use these data. The criteria of sepsis added to the method.
Changes in the text: We have modified our text as advised (see Page 4, line 131-132).

Comment 2: The authors may have included genes that are not really autophagy-related genes such as GAPDH which is a glycolysis-related gene and is not autophagy-related gene. Similarly, many of the genes that are listed in Supplemental Table 1 are not autophagy-related genes i.e. not involved in the formation of autophagosomes or directly regulating the expression of these genes such as.
Reply 2: In our study, ARGs were obtained from the Human Autophagy Database (HADb; http://www.autophagy.lu) and the Molecular Signatures Database v. 7.1 (MSigDB; https://www.gsea-msigdb.org/gsea/msigdb/index.jsp). These databases and methods have been applied in many studies. GAPDH is a glycolysis-related gene and a housekeeping gene, but studies have shown that GAPDH plays a role in the occurrence of autophagy(1,2). At the same time, studies have also demonstrated the role of FOXO1 in autophagy(3).
Changes in the text: None.

Comment 3: Many of the figures are of low resolution and hence the data that they display are not very useful and can not be reproduced. This is particularly true to Figure 1B, Figure 3A, Figure 6B,D, and Figure 9. Figure 9 is very important but the networks shown are not very clear.
Reply 3: The figures have been replaced with the original high-definition version and the compression during image production has been reduced. All experimental results were reproduced because all data sources and experimental methods were open source. Thank you for your suggestion.
Changes in the text: Figure 1-9.

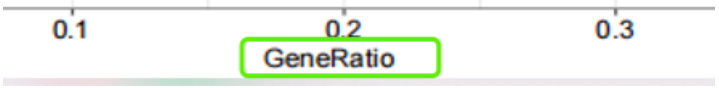
Reviewer D

1. Figure 1:
1) Figure 1 is not clear enough. Please resubmit it in higher resolution.
2) Your Figure 1 legend is wrong. Red dots should be up and green dots should be down.
3) It's suggested to revise the two groups' names to lowercase.

Reply: We have revised the article as required.

2. Figure 2:

1) Please revise all “GeneRatio” to “Gene Ratio”.



2) Please indicate the full name of “GO”, “KEGG”, “BP”, “CC”, “MF” in the legend.

Reply: We have revised the article as required.

3. Figure 3:

Figure 3 is not clear enough. Please resubmit it in higher resolution.

Reply: We have revised the article as required.

4. Figure 4:

Please indicate the full name of “GO”, “BP”, “CC”, “MF” in the legend.

Reply: We have revised the article as required.

5. Figure 5:

Please check Figure 5 legend. There is no * in Figure 5, but you indicated in the legend.

675 set **(C)** and GSE95233 data set **(D)**. ROC, receiver operating characteristic. *P<0.05,
676 **P<0.01, ***P<0.001, ****P<0.0001.

Reply: We have revised the article as required.

6. Figure 6:

1) Figure 6 is not clear enough. Please resubmit it in higher resolution.

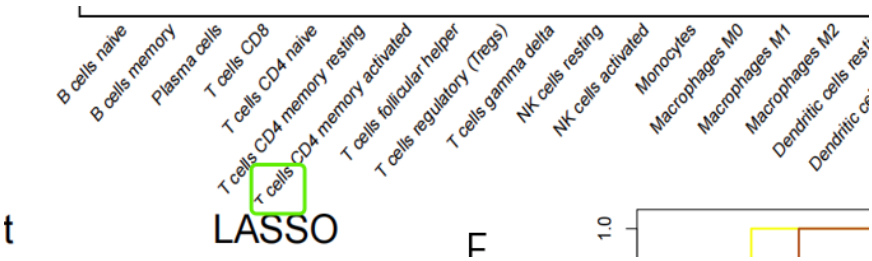
2) Please check Figure 6 legend. There is no **** in Figure 6, but you indicated in the legend.

681 **(A,B)** and the GSE95322 data set **(C,D)** is shown in a histogram and a heatmap.
682 *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

Reply: We have revised the article as required.

7. Figure 7:

There is a word not completed in Figure 7B. Please revise.



8. Figure 8-9 are not clear enough. Please resubmit them in higher resolution.

Reply: We have revised the article as required.