Profiles of immune status and related pathways in sepsis: evidence based on GEO and bioinformatics

HANYU SHEN1; SHIQI REN2; WEI WANG2; CHENLIN ZHANG3; HAIYAN HAO4; QIUYAN SHEN5; YINONG DUAN1; ZIHENG WANG2,6,*; WENLIANG GE7,*

1 Department of Pathogen Biology, School of Medicine, Nantong University, Nantong, 226001, China
2 Department of Clinical Biobank, Affiliated Hospital of Nantong University, Nantong, 226000, China
3 Department of Orthopedics, Qidong Hospital of Chinese Medicine, Nantong, 226200, China
4 Department of Outpatient, Affiliated Hospital of Nantong University, Nantong, 226200, China
5 Department of Neurosurgery, Wuxi Clinical College of Anhui Medical University, 904th Hospital of Joint Logistic Support Force of PLA, Wuxi, 214000, China
6 Department of Neurosurgery, Affiliated Hospital of Nantong University, Nantong, 226200, China
7 Department of Pediatric Surgery, Nantong University Affiliated Hospital, Nantong, 226200, China

Key words: Sepsis, GEO, Bioinformatics, Neutrophil, CD8+ T cell

Abstract: Sepsis, characterized as life-threatening sequential organ failure, is caused by a dysregulated host immune response to a pathogen. Conventional practice for sepsis is to control the inflammation source and administer high-grade antibiotics. However, the mortality rate of sepsis varies from 25~30% and can reach 50% if a septic shock occurs. In our current study, we used bioinformatics technology to detect immune status profiles in sepsis at the genomic level. We downloaded and analyzed gene expression profiles of GSE28750 from the Gene Expression Omnibus (GEO) database to determine differential gene expression and immune status between sepsis and normal samples. Next, we used the CIBERSORT method to quantify the proportions of immune cells in the sepsis samples. Then we explored the differentially expressed genes (DEGs) related to sepsis. Furthermore, gene ontology (GO) function and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were used to present potential signaling pathways in sepsis. We found that in the sepsis samples, the CD8+ T cell fraction was consistently lower, based on the CIBERSORT method, whereas the neutrophil fraction was significantly higher in the sepsis samples. The GO function and KEGG pathway enrichment analysis identified 1573 DEGs that were significantly associated with neutrophil activation, neutrophil degranulation, neutrophil activation involved in the immune response, neutrophil-mediated immunity, and T cell activation in the biological processes group. In our study, we provided a first glance of associations between immune status and sepsis. Furthermore, our data regarding the reciprocal interaction between immune cells (neutrophils and CD8+ T cells) could improve our understanding of immune status profiles in sepsis. However, additional investigations should be performed to verify their clinical value.

Introduction

Sepsis, a complex life-threatening organ dysfunction that ranks as the 10th leading cause of death, is a perplexing imbalance between a pathogen and the body’s immune response (Porte et al., 2019; Verdonk et al., 2017). It was reported that the rapidly increasing incidence of severe bloodstream infections with multidrug-resistant (MDR) pathogens have caused higher health care burdens for governments worldwide (Dalhoff et al., 2018). Sepsis not only causes primary infectious injury but also secondary damage to the infected tissues. Studies have been performed to examine the possible systemic effects of intensive sepsis that leads to the most severe consequence of septic shock, which causes significant morbidity and mortality (Muller-Redetzky, 2017; Osborn, 2017; Singer et al., 2016).

Currently, there are few molecular-based immunotherapies in existence for septic patients (Schrijver et al., 2019). In clinical practice for sepsis, the first step is to control the inflammation source and administer high-grade antibiotics (Liu et al., 2017; Sterling et al., 2015). Furthermore, vital organ support and even resuscitation may also be required.
for severe consequences (Busani et al., 2017). In the past few years, clinical trials from some large institutions have proven to be disappointing because of the complex heterogeneity of study populations and immunological phenotypes (Peters Van Ton et al., 2018). To date, researchers have explored immunosuppressive avenues for the treatment of sepsis, which leads to striking morbidity and mortality caused by sepsis-induced immunoparalysis (Bruse et al., 2019; Zijlstra et al., 2019). However, the current therapeutic focus has shifted from immunosuppressive strategies to enhancing the host’s immune response (Esposito et al., 2017; Hagel et al., 2019).

It is well known that the initial immune response to infection is mounted by host cellular and humoral mediators, while neutrophils, as early responding immune cells, are recruited to the site of infection to exert their functions (Liu and Sun, 2019). However, recent studies showed that neutrophils may in fact be a double-edged sword in sepsis that could induce pyroptosis to fulfill their role in the active immune response. Therefore, it is crucial that we should pay close attention to the regulation of neutrophils when dealing with sepsis clinically.

Thus, in our current study, we used bioinformatics technology to detect immune status profiles in sepsis at the genome level. The Gene Expression Omnibus (GEO) database offers a pioneering medium of the genomic events in large cohorts worldwide, which serves as a public repository for archiving high-throughput microarray experimental data. We downloaded and analyzed the gene expression profiles of GSE28750 from the GEO database to determine the differential gene expression and immune status between sepsis and normal samples.

Materials and Methods

Data resources
The differentially expressed genes (DEGs) and the immune status during sepsis were investigated, relative to normal samples, after downloading and analyzing GSE28750 (Sutherland et al., 2011) profiles from the GEO database (Clough and Barrett, 2016) (http://www.ncbi.nlm.nih.gov/geo/) that essentially serves as a public repository, wherein high-throughput microarray experimental data is archived. The platform of GSE28750 was GPL 570 (Affymetrix Human Genome U113 Plus 2.0 Array).

Estimation of immune cell fractions
The well-designed CIBERSORT method (Newman et al., 2019) (http://cibersort.stanford.edu/), validated on gene expression profiles measured using microarrays, helped quantify the immune cell proportions in sepsis samples. CIBERSORT comprises 547 genes and specifically facilitates highly sensitive discrimination of 22 human hematopoietic cell phenotypes, including B cells, T cells, natural killer cells, macrophages, dendritic cells, and myeloid subsets. CIBERSORT established a P-value via the Monte Carlo method for deconvolution of each sample, offering a measure of confidence in our results, wherein the fractions of immune cell populations inferred at a threshold of $<0.05$ were considered accurate (Newman et al., 2015), and only patients conforming to this were then considered eligible for further investigation. The immune cell proportions were individually projected for each gene expression series, so for each sample, the sum of all estimates equaled 1.

Identification of DEGs
The downloaded original files were cataloged into sepsis and normal groups. The Bioconductor package ‘affy’ (http://www.bioconductor.org/) standardized and transformed raw data into expression values (Gautier et al., 2004). The DEGs between early-detection sepsis and normal tissue samples were identified via applying a significance analysis of the empirical Bayes method within the Limma package (Ritchie et al., 2015). Adj. P-value $< 0.01$ and logFC $> 1$ were the designated cut-off criteria to select significant DEGs.

Functional enrichment analysis
R language clusterProfiler package enrichment analysis facilitated the analysis of potential biological processes (BP), cellular components (CC) and molecular functions (MF) related to DEGs (Ashburner et al., 2000; Pickett and Edwardson, 2006; Yu et al., 2012). A KEGG pathway enrichment analysis presented potential signaling pathways. KEGG, as a comprehensive resource to ascertain functional and metabolic pathways, comprises exhaustive database compilations with detailed information on genomes, biological pathways, diseases, chemical substances, and drugs (Kanehisa and Goto, 2000; Ogata et al., 1999). A P-value of $<0.05$ was deemed statistically significant.

Results

Estimation of immune cell fractions
The CIBERSORT fractions presented in Fig. 1B revealed CD8+ T cells were consistently lower in sepsis, compared with normal samples, whereas the neutrophil fraction was considerably higher in sepsis samples.

Identification of DEGs
Subsequent to pre-processing, a total of 1573 DEGs were identified in sepsis, relative to control samples. Fig. 2 presents a volcano plot of sepsis DEGs from each dataset.

GO function and KEGG pathway enrichment analysis
R language clusterProfiler, used to apply GO function and KEGG pathway enrichment analysis, offered a detailed insight into DEGs, and the GO results were further categorized functionally to incorporate MF, BP, and CC. For MF, these DEGs were enriched for MHC class II protein binding complex, MHC protein binding complex, cytokine binding, protein tyrosine kinase binding, and protein kinase regulator activity. Moreover, these genes were significantly enriched in specific and tertiary granules, cytoplasmic vesicle lumen, vesicle lumen, and secretory granule lumen in the CC category. In the BP group, these DEGs were significantly associated with neutrophil activation, neutrophil degranulation, neutrophil activation involved in immune response, neutrophil-mediated immunity, and T cell activation (Fig. 3 and Tab. 1). The results of the KEGG pathway analysis showed that DEGs were mainly enriched in pathways in the hematopoietic cell lineage, Th1 and Th2 cell differentiation, Th17 cell differentiation, inflammatory bowel disease (IBD), programmed death (PD) ligand 1
expression and the PD-1 checkpoint pathway in cancer, human T-cell leukemia virus 1 infection, the T cell receptor signaling pathway, primary immunodeficiency, Epstein-Barr virus infection and leishmaniasis (Fig. 4 and Tab. 2).

Discussion

Sepsis, characterized as life-threatening sequential organ failure, is caused by a dysregulated host immune response to a pathogen (Pei et al., 2018). It is vital that a balanced host immune response is maintained to eliminate systemic inflammatory responses and restore sequential organ functions. However, the underlying evolutionary mechanisms of host sepsis-induced inflammation, immunosuppression, and organ failure remain unknown (Drigo et al., 2018). Some immune modulators, such as Thymosin alpha 1 (Tα1), have been employed to great biological effect for septic patients with systemic inflammatory response syndrome (Pei et al., 2018; Pica et al., 2018). Although Tα1 seems to serve as an important alternative therapy supporting treatment for sepsis in these previous studies, sepsis manifests diversely, including systemic inflammatory response syndrome, and so identical treatment is not appropriate for all septic patients. Nevertheless, it is understood that there are powerful links between activation of first-line immune cells and the immunopathogenesis of sepsis (Kumar, 2018). Experiments investigating dysregulated activation of immune cells during sepsis progression could provide promising targets for immunomodulatory therapy.

In order to seek potential targets for immunomodulatory therapy, we have provided a first glance of associations between immune status and sepsis. In our current study, we first downloaded and analyzed the gene expression profiles of GSE28750 from the GEO database to investigate the

FIGURE 1. (A) Differences in immune status between normal and sepsis samples. (B) Box plot of 22 immune cells in normal and sepsis samples.
differential gene expression and immune status between sepsis and normal samples. Next, we used the CIBERSORT method to quantify the proportions of immune cells in the sepsis samples and detected highly sensitive and specific discrimination of 22 human hematopoietic cell phenotypes, including B cells, T cells, natural killer cells, macrophages, dendritic cells, and myeloid subsets. Significance analysis by the empirical Bayes methods within the Limma package was then applied to identify DEGs between early detection of sepsis samples and control samples based on the original CEL files. We identified a total of 1573 DEGs in sepsis samples compared with normal tissue samples, and the fractions of CD8+ T cells were consistently lower as determined by CIBERSORT, whereas the fractions of neutrophils were significantly higher in the
sepsis samples. Furthermore, GO function and KEGG pathway enrichment analysis found that these 1573 DEGs were significantly associated with neutrophil activation, neutrophil degranulation, neutrophil activation involved in the immune response, neutrophil-mediated immunity, and T cell activation in the BP group.
During the first stage of the body’s innate response to infection, neutrophils which serve as early responders play a key role in adaptive immune response progress, which includes anti-microbial CD4+ and CD8+ T-cell responses. It is well established that a reciprocal relationship exists between neutrophils and T cells, with neutrophils suppressing T cell activation. Research has revealed that neutrophils, by releasing reactive oxygen species, myeloperoxidase, and arginase to exert their effects, can suppress human T cell activation in vitro (El-Hag et al., 1986). A similar phenomenon of neutrophil-mediated T cell inhibition can be observed in both tumor patients and normal pregnancy. Recent research has found that with the increasing proportions of neutrophils, T cell function was remarkably reduced because of increasing arginase-1 levels in glioma patients (Kropf et al., 2007). Likewise, during normal pregnancy, it was found that the higher levels of arginase-1 expressed by neutrophils in the placenta and maternal blood were associated with T cell hyporesponsiveness. In our study, we identified that CD8+ T cell fractions were consistently lower in sepsis samples, while the neutrophil fraction was significantly higher in the sepsis samples.

In conclusion, we suggest a comprehensive estimate of associations between inflammatory response and sepsis. The fractions of both CD8+ T cells and neutrophils could improve our understanding of the heterogeneity of sepsis that promotes the immune status profiles in sepsis. More experiments are required to detect the reciprocal relationship between neutrophils and CD8+ T cells to elucidate the mechanism of action and identify prospective insights during sepsis progression.

Acknowledgement: We thank Gillian Campbell, Ph.D., from Liwen Bianji, Edanz Group China (www.liwenbianji.cn/ac), for editing the English text of a draft of this manuscript.

Availability of Data and Materials: The following information was supplied regarding data availability: The raw data was downloaded from the publicly available GEO database (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE28750).

Table 2

| ID          | Description                                      | P-value |
|-------------|--------------------------------------------------|---------|
| hsa04640    | Hematopoietic cell lineage                       | 2.27E-14|
| hsa04658    | Th1 and Th2 cell differentiation                 | 3.56E-14|
| hsa04659    | Th17 cell differentiation                        | 6.16E-14|
| hsa05321    | Inflammatory bowel disease (IBD)                 | 4.18E-09|
| hsa05235    | PD-L1 expression and PD-1 checkpoint pathway in cancer | 4.25E-08 |
| hsa05166    | Human T-cell leukemia virus 1 infection          | 2.54E-07|
| hsa04660    | T cell receptor signaling pathway                | 4.26E-07|
| hsa05340    | Primary immunodeficiency                         | 9.12E-07|
| hsa05169    | Epstein-Barr virus infection                     | 1.01E-06|
| hsa05140    | Leishmaniasis                                     | 1.25E-06|

Funding Statement: Jiangsu Modern Hospital Management Research Fund (JSY-3-2019-053), Postgraduate Research & Practice Innovation Program of Jiangsu Province (No. KYCX20_2839).

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

References

Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, Harris MA, Hill DP, Issel-Tarver L, Kasarskis A, Lewis S, Matese JC, Richardson JE, Ringwald M, Rubin GM, Sherlock G (2000). Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nature Genetics 25: 25–29. DOI 10.1038/75556.

Bruse N, Leijte GP, Pickers P, Kox M (2019). New frontiers in precision medicine for sepsis-induced immunoparalysis. Expert Review of Clinical Immunology 15: 251–263. DOI 10.1080/1744666X.2019.1562336.

Busani S, Roat E, Serafini G, Mantovani E, Biagioni E, Girardis M (2017). The role of adjunctive therapies in septic shock by gram negative MDR/XDR infections. Canadian Journal of Infectious Diseases and Medical Microbiology 2017: 2808203. DOI 10.1155/2017/2808203.

Clough E, Barrett T (2016). The gene expression omnibus database. Methods in Molecular Biology 1418: 93–110. DOI 10.1007/978-1-4939-3578-9_5.

Dalhoff K, Abele-Horn M, Andreas S, Deja M, Ewig S, Gastmeier P, Gatermann S, Gerlach H, Grabein B, Heussel CP, Hoffken G, Kolditz M, Kramme E, Kuhl H, Lange C, Mayer K, Nachtigall I, Panning M, Pletz M, Rohde G, Scherke M, Schutte H, Seifert H, Spies C, Welte T (2018). Epidemiology, diagnosis and treatment of adult patients with nosocomial pneumonia-update 2017-53. Guideline of the German Society for Anaesthesiology and Intensive Care Medicine, the German Society for Infectious Diseases, the German Society for Hygiene and Microbiology, the German Respiratory Society and the Paul-Ehrlich-Society for Chemotherapy, the German Radiological Society and the German Society for Virology. Pneumologie 72: 15–63. DOI 10.1055/s-0043-121734.
Drigo M, Giacomini E, Lazzaro M, Pasotto D, Bilato D, Ruggeri J, Boniotti MB, Alboralí GI, Amadoti M (2018). Comparative evaluation of immune responses of swine in PRRS-stable and unstable herds. *Veterinary Immunology and Immunopathology* **200**: 32–39. DOI 10.1016/j.vetimm.2018.04.007.

El-Hag A, Lipsky PE, Bennett M, Clark RA (1986). Immunomodulation by neutrophil myeloperoxidase and hydrogen peroxide: differential susceptibility of human lymphocyte functions. *Journal of Immunology* **136**: 3420–3426.

Esposito S, De Simone G, Boccia G, De Caro F, Pagliano P (2017). Sepsis and septic shock: New definitions, new diagnostic and therapeutic approaches. *Journal of Global Antimicrobial Resistance* **10**: 204–212. DOI 10.1016/j.jgar.2017.06.013.

Gautier L, Cope L, Bolstad BM, Irizarry RA (2004). affy - analysis of Affymetrix GeneChip data at the probe level. *Bioinformatics* **20**: 370–377. DOI 10.1093/bioinformatics/btg405.

Hagel S, Fiedler S, Hohn A, Brinkmann A, Frey OR, Hoyer H, Schlettmann P, Kiehntopf M, Roberts JA, Pletz MW (2019). Therapeutic drug monitoring-based dose optimisation of piperacillin/tazobactam to improve outcome in patients with sepsis (TARGET): a prospective, multi-centre, randomised controlled trial. *Trials* **20**: 330. DOI 10.1186/s13063-019-3437-x.

Kanehisa M, Goto S (2000). KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Research* **28**: 28–30. DOI 10.1093/nar/28.1.27.

Kropf P, Baud D, Marshall SE, Munder M, Mosley A, Fuentes JM, Bangham CR, Taylor GP, Herath S, Choi BS, Soler G, Teoh T, Modolell M, Muller I (2007). Arginase activity mediates reversible T cell hyporesponsiveness in human pregnancy. *European Journal of Immunology* **37**: 935–945. DOI 10.1002/eji.200636542.

Kumar V (2018). Targeting macrophage immunomodulation: dawn in the darkness of sepsis. *International Immunopharmacology* **58**: 173–185. DOI 10.1016/j.intimp.2018.03.005.

Liu VX, Fielding-Singh V, Greene JD, Baker JM, Iwashyna TJ, Bhattacharya J, Escobar GJ (2017). The timing of early antibiotics and hospital mortality in sepsis. *American Journal of Respiratory and Critical Care Medicine* **196**: 856–863. DOI 10.1164/rccm.201609-1848OC.

Liu L, Sun B (2019). Neutrophil pyroptosis: new perspectives on sepsis. *Cellular and Molecular Life Sciences* **76**: 2031–2042. DOI 10.1007/s00018-019-0360-0.

Muller-Redetzky H (2017). Sepsis and septic shock: Overview after sepsis-3 and the requirements of the clinician regarding the autopsy of critically ill patients. *Pathology* **38**: 365–369. DOI 10.1007/s00292-017-0301-0.

Newman AM, Liu CL, Green MR, Gentles AJ, Feng W, Xu Y, Hoang CD, Diehn M, Alizadeh AA (2015). Robust enumeration of cell subsets from tissue expression profiles. *Nature Methods* **12**: 453–457. DOI 10.1038/nmeth.3357.

Newman AM, Steen CB, Liu CL, Gentles AJ, Chaudhuri AA, Scherer F, Khodadoust MS, Esfahani MS, Luca BA, Steiner D, Diehn M, Alizadeh AA (2019). Determining cell type abundance and expression from bulk tissues with digital cytometry. *Nature Biotechnology* **37**: 773–782. DOI 10.1038/s41587-019-0114-2.

Ogata H, Goto S, Sato K, Fujibuchi W, Bono H, Kanekawa M (1999). KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Research* **27**: 29–34. DOI 10.1093/nar/27.1.29.

Osborn TM (2017). Severe sepsis and septic shock trials (ProCESS, ARISE, ProMISE): what is optimal resuscitation? *Critical Care Clinics* **33**: 323–344. DOI 10.1016/j.ccc.2016.12.004.

Pei F, Guan X, Wu J (2018). Thymosin alpha 1 treatment for patients with sepsis. *Expert Opinion on Biological Therapy* **18**: 71–76. DOI 10.1080/14712598.2018.1484104.

Peters Van Ton AM, Kox M, Abdo WF, Pickkers P (2018). Precision immunotherapy for sepsis. *Frontiers in Immunology* **9**: 1926. DOI 10.3389/fimmu.2018.01926.

Pica F, Gaiazzoni R, Casaliniuova I, Moroni G, Bue C, Limongi D, D’agostini C, Tomino C, Perricone R, Palamara AT, Sinibaldi Vallebona P, Garaci E (2018). Serum thymosin alpha 1 levels in normal and pathological conditions. *Expert Opinion on Biological Therapy* **18**: 13–21. DOI 10.1080/14712598.2018.1474197.

Pickett JA, Edwardsion JM (2006). Compound exocytosis: mechanisms and functional significance. *Traffic* **7**: 109–116. DOI 10.1111/j.1600-0854.2005.00372.x.

Porte R, Davoudian S, Agari F, Parente R, Mantovani A, Garlanda C, Bottazzi B (2019). The long pentraxin PTX3 as a humoral innate immunity functional player and biomarker of infections and sepsis. *Frontiers in Immunology* **10**: 794. DOI 10.3389/fimmu.2019.00794.

Ritchie ME, Pipson B, Wu D, Hu Y, Law CW, Shi W, Smyth GK (2015). limma powers differential expression analyses for DNA-amplification and microarray studies. *Nucleic Acids Research* **43**: e47. DOI 10.1093/nar/gkv007.

Schrijver IT, Theroude C, Roger T (2019). Myeloid-derived suppressor cells in sepsis. *Frontiers in Immunology* **10**: 327. DOI 10.3389/fimmu.2019.00327.

Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, Bellomo R, Bernard GR, Chiche JD, Coopersmith CM, Hotchkiss RS, Levy MM, Marshall JC, Martin GS, Opal SM, Rubenfeld GD, Van Der Poll T, Vincent JL, Angus DC (2016). The third international consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA* **315**: 810–810. DOI 10.1001/jama.2016.0287.

Sterling SA, Miller WR, Pryor J, Puskarich MA, Jones AE (2015). The impact of timing of antibiotics on outcomes in severe sepsis and septic shock: a systematic review and meta-analysis. *Critical Care Medicine* **43**: 1907–1915. DOI 10.1097/ CCM.0000000000001142.

Sutherland A, Thomas M, Brandon RA, Brandon RB, Lipman J, Tang B, Mclean A, Pascoe R, Price G, Nguyen T, Stone G, Venter D (2011). Development and validation of a novel molecular biomarker diagnostic test for the early detection of sepsis. *Critical Care* **15**: R149. DOI 10.1186/cc10274.

Verdonk F, Blet A, Mebazaa A (2017). The new sepsis definitions: limitations and contribution to research and diagnosis of sepsis. *Current Opinion in Anaesthesiaesthesia* **30**: 200–204. DOI 10.1097/AOC.0000000000000446.

Yu G, Wang LG, Han Y, He QY (2012). clusterProfiler: an R package for comparing biological themes among gene clusters. *Omics: A Journal of Integrative Biology* **16**: 284–287. DOI 10.1089/ omi.2011.0118.

Zijlstra JG, Van Meurs M, Moser J (2019). Commentary: precision immunotherapy for sepsis. *Frontiers in Immunology* **10**: DOI 10.3389/fimmu.2019.00020.