BIOCELL
2020 44(4): 583-589

Tech Science Press

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

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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

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Received: 02 May 2020; Accepted: 23 June 2020

Doi: 10.32604/biocell.2020.011345

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

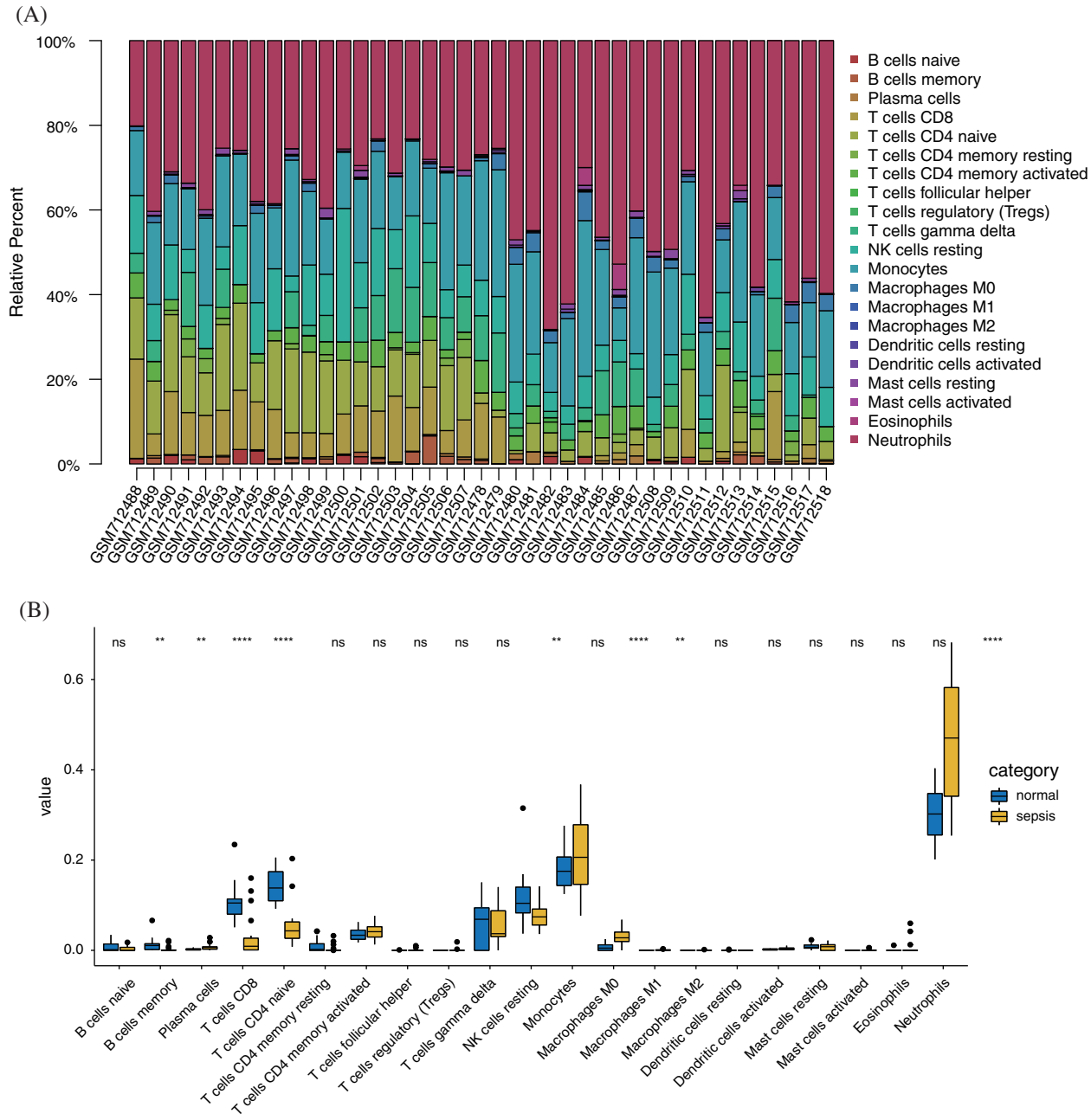

FIGURE 1. (A) Differences in immune status between normal and sepsis samples. (B) Box plot of 22 immune cells in normal and sepsis samples.

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

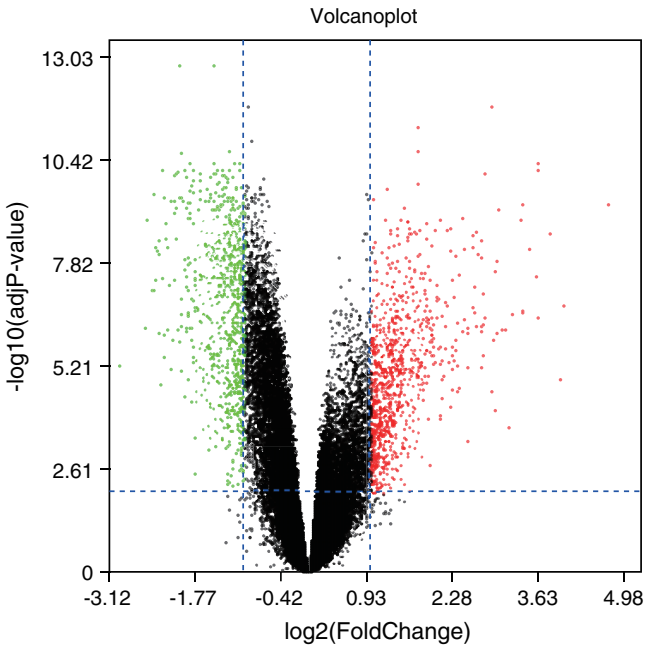

FIGURE 2. Volcano plot of DEGs between sepsis and normal samples. Red dots: significantly up-regulated genes in sepsis; green dots: significantly down-regulated genes in sepsis; black dots: non-differentially expressed genes.

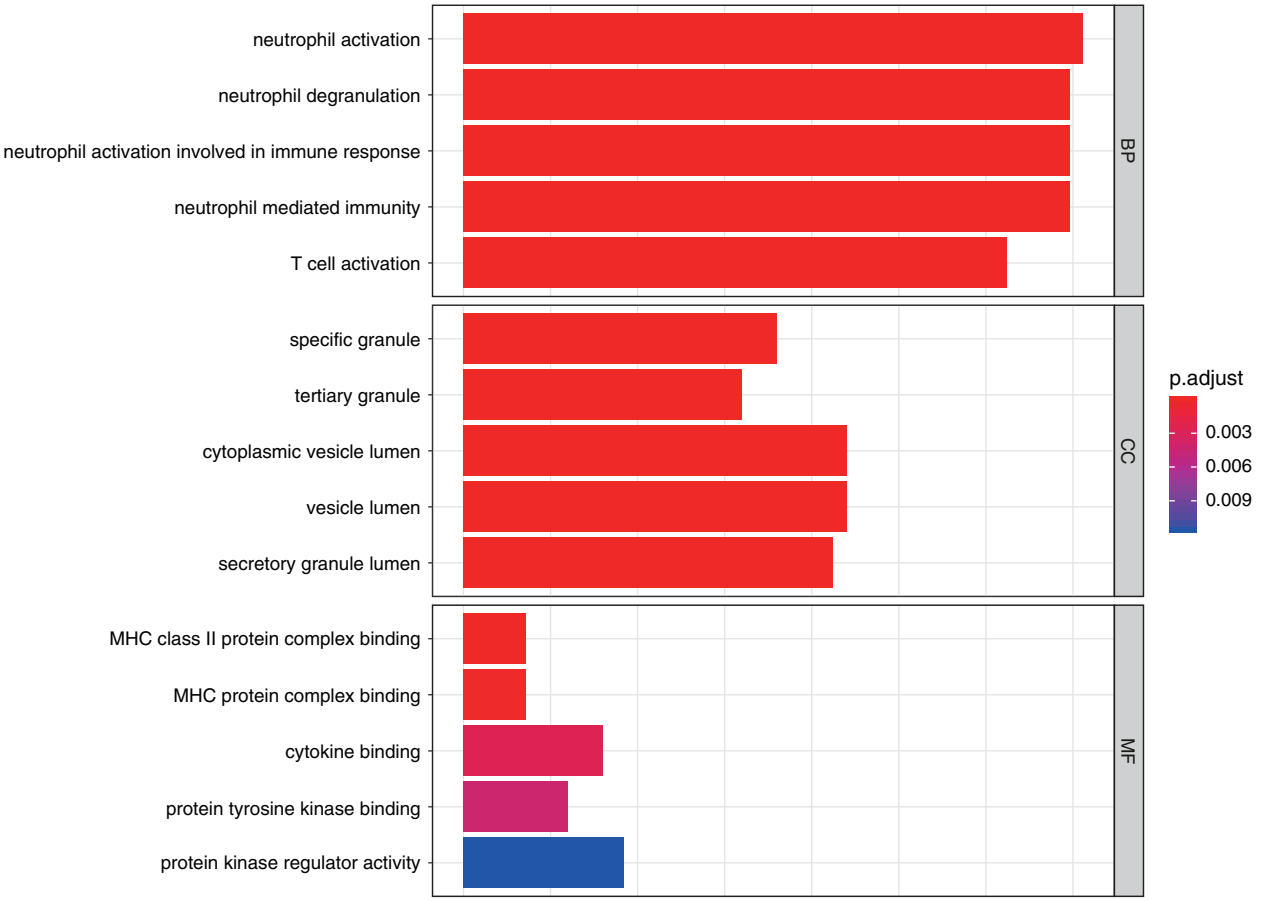

FIGURE 3. GO analysis of the DEGs in sepsis, including biological processes, cellular components, and molecular function.

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

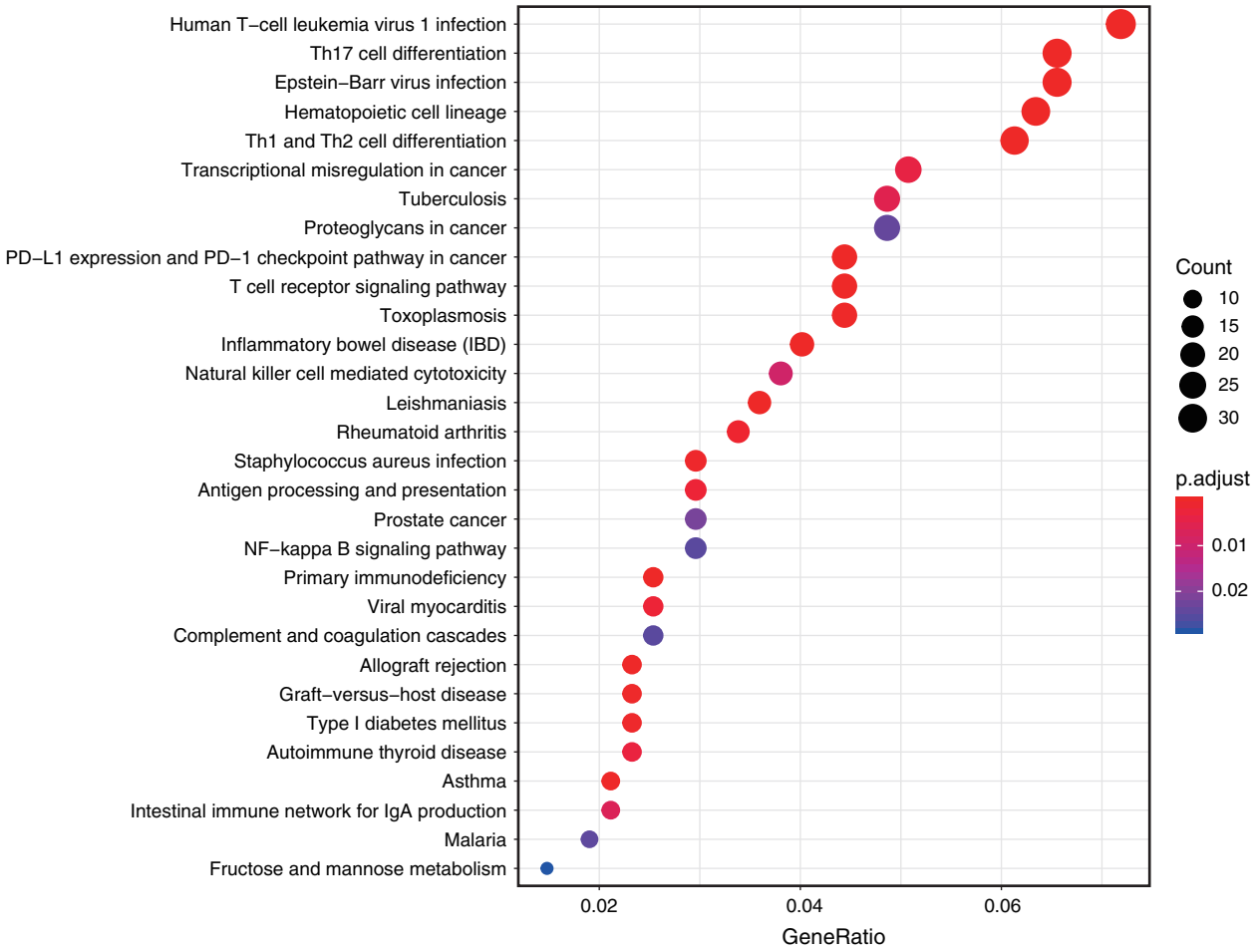

FIGURE 4. KEGG pathway enrichment of sepsis.

TABLE 1

GO enrichment analysis of the DEGs

ONTOLOGY	ID	Description	*P*-value
BP	GO:0042119	neutrophil activation	5.90E-26
BP	GO:0043312	neutrophil degranulation	1.67E-25
BP	GO:0002283	neutrophil activation involved in immune response	2.61E-25
BP	GO:0002446	neutrophil mediated immunity	1.30E-24
BP	GO:0042110	T cell activation	6.35E-22
CC	GO:0042581	specific granule	2.35E-22
CC	GO:0070820	tertiary granule	1.34E-17
CC	GO:0060205	cytoplasmic vesicle lumen	3.05E-15
CC	GO:0031983	vesicle lumen	3.47E-15
CC	GO:0034774	secretory granule lumen	5.28E-15
MF	GO:0023026	MHC class II protein complex binding	2.49E-08
MF	GO:0023023	MHC protein complex binding	3.01E-07
MF	GO:0019955	cytokine binding	8.38E-06
MF	GO:1990782	protein tyrosine kinase binding	1.88E-05
MF	GO:0019887	protein kinase regulator activity	7.65E-05

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.

TABLE 2

KEGG pathway enrichment analysis of DEGs

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

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).

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.

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