


## Research Article

# Insights from the Analysis of the Sepsis Dataset: Understanding the Immune Dynamics and Molecular Pathways in Sepsis

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Sepsis, a critical medical condition instigated by infections, profoundly alters molecular and cellular immune responses. This study focused on the GSE54514 dataset obtained from the GEO database to uncover the complex gene expression patterns and related pathways in sepsis. A total of 42 genes were found to be expressed differently in septic patients compared to those of healthy individuals. The enrichment analyses of pathways revealed the disruption of natural immune pathways such as toll-like receptor signaling, regulation of actin cytoskeleton, and NOD-like receptor signaling. Through ssGSEA analysis, we revealed a strong association between sepsis and immune cell dynamics, finding significant correlations with HLA-related genes and distinct immune cell populations. Furthermore, genes such as CCL5, CD274, CD3E, and CD8A were linked with pathways, notably the “ribosome” pathway, suggesting potential roles in sepsis-related immune responses. The extensive examination provides fresh perspectives on the gene expression and pathway changes in sepsis, laying the groundwork for upcoming therapeutic interventions and a more profound comprehension of this intricate condition.

## 1. Introduction

For a considerable amount of time, sepsis has been a significant worldwide health issue, causing life-threatening dysfunction of organs due to uncontrolled host reactions to infection. Sepsis imposes a significant load, with approximations suggesting its impact on millions of individuals globally each year, resulting in a considerable portion of admissions to intensive care units. Its high prevalence has made it a critical focus for healthcare professionals and researchers alike. From an epidemiological standpoint, the incidence of sepsis has been on the rise although the reasons for this increase are multifaceted [1]. This upward trend has been influenced by various factors, including the aging of populations, the emergence of antibiotic-resistant pathogens, and a growing awareness and recognition of the condition [2]. Despite the rise in incidence, there has been a notable decrease in sepsis-related mortalities in many regions thanks to advances in early detection and prompt medical interventions [3]. Significant

advancements have been made in the field of treatment over the last few decades. The emphasis on early recognition and initiation of broad-spectrum antibiotics, combined with aggressive fluid resuscitation, has been foundational in sepsis management [4]. Moreover, the investigation into the pathogenesis of sepsis has opened doors for specific treatments, centered on regulating the immune system and safeguarding organ functionality [5]. These advances, coupled with improvements in supportive care measures and monitoring, have led to better outcomes for sepsis patients [6].

The immune system, an intricate web of cells, tissues, and biochemical substances, acts as the primary means of protection for the body against pathogens [7]. Its intricate design allows it to distinguish between self and nonself, ensuring protection against a plethora of threats, from bacterial invasions to cancerous cell growth [7]. At the core of this system are the immune cells, which have unique and synchronized functions in identifying, assaulting, and retaining knowledge of external agents [8]. Various types of immune cells, such as

T cells, B cells, macrophages, and dendritic cells, work together to coordinate the immune response of the body. T cells, for instance, are critical for cell-mediated immunity, recognizing and destroying infected or malignant cells [9]. B cells, on the other hand, produce antibodies that can neutralize pathogens. Macrophages and dendritic cells serve as antigen-presenting cells, connecting the innate and adaptive components of the immune system through the processing and display of antigens to T cells [10]. Immunotherapy, also referred to as medical treatments, has been introduced as a new era due to the profound comprehension of these cellular mechanisms. The objective of this method is to utilize the potential of the immune system in fighting illnesses, specifically tumors. By either boosting the body's natural defenses or training them to specifically target disease cells, immunotherapy offers a promising alternative or supplement to traditional treatments such as chemotherapy and radiation [11]. Significant progress in this area encompasses the use of checkpoint inhibitors, which unleash the inhibitions on T cells, enabling them to aggressively target tumors [12]. In addition, CAR-T cell therapy has emerged, involving the genetic modification of patients' T cells to enhance their ability to identify and eliminate cancer cells [13].

Given its rapid advancement and significant fatality rate, sepsis, an infection-induced systemic inflammatory reaction, continues to be a highly complex medical condition to handle. The immune system plays a crucial part in sepsis. While the body's immune response aims to eliminate the invading pathogens, an uncontrolled or exaggerated response can lead to widespread inflammation, multiorgan dysfunction, and eventual failure [14]. This delicate balance between protective immunity and harmful overreaction is pivotal in determining the outcome of sepsis. The complex interplay of immune cells, such as T cells, B cells, macrophages, and dendritic cells, greatly influences the pathophysiology of sepsis [15]. For example, sepsis typically begins with a hyperinflammatory stage that frequently results in an overabundance of cytokines being released, referred to as a "cytokine storm," which has the potential to harm tissues and organs. Following this stage, there is a possibility of entering an immunosuppressed condition, in which the patient's immune system weakens, making them susceptible to additional infections [16]. Nevertheless, the utilization of immunotherapy in septicemia is still in its early stages. The unique challenges arise from the intricate nature of the immune response in sepsis combined with the diverse patient population [17]. As researchers and clinicians continue to unravel the intricate web of immune interactions in sepsis, the hope remains that immunotherapy could provide novel avenues for more effective treatments, improving outcomes for patients afflicted with this devastating condition.

## 2. Methods

**2.1. Data Retrieval from GEO.** GEO, the Gene Expression Omnibus, is an open repository that stores and shares datasets of high-throughput gene expression and other functional genomics. To conduct our study, we obtained gene expression datasets from the GEO database available at <https://www.ncbi.nlm.nih.gov/geo/>.

**2.2. Background Correction and Normalization.** Raw expression data were imported into the R environment. A robust multiarray average (RMA) was used to correct the background. Subsequently, normalization was carried out using the quantile normalization to ensure that the distributions of intensities were consistent across all arrays.

**2.3. Analysis of Differential Expression.** Genes with a  $p$  value less than 0.05 and an absolute fold change greater than 1.2 were considered differentially expressed following pre-processing using the "limma" package in R.

**2.4. Enrichment Analysis of Gene Ontology (GO) and the KEGG Pathway.** The analysis of Gene Ontology (GO) offers a structured and regulated terminology for characterizing the attributes of genes and gene products in all organisms. The three domains include biological process (BP), cellular component (CC), and molecular function (MF). The differentially expressed genes were mapped to GO terms in the database, and Fisher's exact test was used to test for significant enrichment of over-represented GO terms. Significantly enriched terms were determined based on a corrected  $p$  value (following Benjamini-Hochberg correction for multiple testing) below 0.05. KEGG, also known as the Kyoto Encyclopedia of Genes and Genomes, is a comprehensive compilation of databases that encompass genomics, biological pathways, diseases, pharmaceuticals, and chemical compounds. The analysis of KEGG pathways offers valuable information about the possible biological roles and relationships among genes. In order to conduct KEGG pathway analysis, we utilized the R package called "clusterProfiler." The genes that showed differential expression were matched with KEGG pathways, and the detection of significantly enriched pathways was done using Fisher's exact test. Pathways that had a corrected  $p$  value (following Benjamini-Hochberg correction for multiple testing) below 0.05 were considered to be significantly over-represented. With the help of the R package "enrichplot," we generated dot plots and bar plots to visualize our GO and KEGG enrichment analyses. These plots provided a graphical representation of the enriched terms and pathways, displaying the gene ratio and significance level for each term and pathway.

**2.5. The Analysis of Immune Cell Infiltration Using Multiple Methods.** The presence of immune cells in the tumor microenvironment (TME) is crucial for the advancement of cancer and its reaction to therapies. Measuring the comparative prevalence of different immune cell categories in the tumor microenvironment (TME) can offer valuable understanding of tumor immunology and possible therapeutic approaches. For this research, we utilized various algorithms to accurately determine the presence of immune cells based on the data of gene expression. CIBERSORT is an approach that utilizes a collection of reference gene expression values (known as a signature matrix) to approximate the proportions of different cell types within mixed cell populations.

Using the LM22 signature matrix, we utilized CIBERSORT on our gene expression data that had been normalized. This matrix has the ability to differentiate between 22 phenotypes of human immune cells. Results that had a deconvolution  $p$  value below 0.05 were deemed trustworthy and kept for additional examination. TIMER serves as a comprehensive tool for the systematic examination of immune infiltrates in various types of cancer. The abundance of six immune infiltrates, including B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages, and dendritic cells, was inferred using the TIMER algorithm. Abundance scores were normalized across samples for comparison. quanTIseq is a comprehensive pipeline that calculates the proportions of ten immune cell populations based on gene expression profiles of tumors. Following the suggested preprocessing of the gene expression data, we executed quanTIseq to acquire estimations of immune cell fractions for every sample.

**2.6. GSEA (Gene Set Enrichment Analysis).** We made use of GSEA software provided by the Broad Institute for our research. The dataset of gene expression was sorted according to the correlation between the expression of each gene and the phenotype label. Afterwards, a calculation was performed to determine the enrichment score (ES) for every gene set, indicating the extent to which a gene set is over-represented at either the top or bottom of the list that has been ranked. In this study, the importance of the ES was evaluated using a permutation test, with 1,000 permutations conducted. To consider the gene set's size, the calculated normalized enrichment score (NES) was used. Gene sets that had a false discovery rate (FDR) below 0.25 and a nominal  $p$  value below 0.05 were deemed as significantly enriched.

**2.7. Analysis of Gene Set Variation (GSVA).** The GSVA method was utilized with the R package called "GSVA." Inputting the gene expression data, we computed enrichment scores for each sample using gene sets obtained from the Molecular Signatures Database (MSigDB). The resulting matrix, which represents the enrichment scores of gene sets in different samples, was utilized in subsequent analyses for differential enrichment.

### 3. Results

**3.1. Sepsis May Involve Potential Genes and Pathways That Exhibit Differential Expression.** To investigate the potential genes that are strongly linked to sepsis, the GSE54514 dataset from the GEO database was incorporated in this study. The study included a group of 18 individuals who were healthy, as well as 26 individuals who survived sepsis and 9 individuals who did not survive sepsis in the dataset GSE54514 (Figure 1(a)). Initially, we conducted a comparative analysis of gene expression between individuals without any health issues and individuals diagnosed with sepsis. Subsequently, a grand total of 42 genes were recognized as the genes with differential expression, encompassing 21 genes that were downregulated and 21 genes that were upregulated (Figures 1(b) and 1(c)). In addition, we conducted an

analysis to enrich the pathways. The KEGG enrichment analysis revealed that the toll-like receptor signaling pathway, regulation of actin cytoskeleton, NOD-like receptor signaling pathway, and RIG-1 like receptor signaling pathway exhibited the highest levels of enrichment in the results (Figures 2(a) and 2(b)). Furthermore, regarding the GO enrichment analysis, the findings indicated that the most associated pathways were regulation of cellular senescence, regulation of cell aging, inhibition of T cell activation, activation of macrophages, coagulation, and cellular response to type I interferon (Figures 2(c) and 2(d)).

**3.2. The Analysis of ssGSEA Uncovered the Association between Sepsis and Cells Related to the Immune System.** Initially, to assess the involvement of immune-related cells in the sepsis group, we conducted ssGSEA analysis to determine the immune-related scores for every sepsis patient. Subsequently, utilizing the immune-associated scores, individuals diagnosed with sepsis were categorized into groups with low and high immune-related scores (Figure 3(a)). Subsequently, the t-SNE (t-distributed stochastic neighbor embedding) examination showcased the dispersion of individuals afflicted with sepsis (Figure 3(b)). Moreover, the heatmap exhibited the ratings for cells associated with the immune system, pathways associated with the immune system, and functions associated with the immune system in the sepsis group (Figure 3(c)). Next, we conducted a correlation analysis to assess the relationship between immune-related scores and genes associated with human leukocyte antigen (HLA). The findings indicated that the levels of HLA-DMB, HLA-DPA1, HLA-DPB1, and HLA-DQA1 expression exhibit a positive correlation with the immune-related environment (Figure 3(d)). Furthermore, we conducted a correlation analysis between immune-related cells and immune-related scores in the sepsis cohort. In the sepsis cohort, there was a notable association between the immune-related score and memory B cells, inexperienced B cells, CD8 T cells, inactive NK cells, M0 macrophages, and neutrophils.

**3.3. Discovering the Crucial Genes Associated with Sepsis and Functions Related to the Immune System.** Based on the ssGSEA analysis, the sepsis cohort was effectively categorized into groups with low and high immune-related characteristics. Afterwards, we conducted the analysis of gene expression differences between groups with low and high immune activity (Figure 4(a)). The findings indicated that a sum of 83 genes were identified as the genes with differential expression, comprising of 18 genes that were downregulated and 65 genes that were upregulated (Figure 4(b)). Moreover, the Venn diagram indicated that 12 genes showed a strong correlation with immune-related activities and sepsis (Figure 4(c)).

**3.4. The Pathways Enrichment Analysis Revealed the Key Pathways in the Sepsis Cohort.** In the GSEA analysis for the gene CCL5, several key pathways were identified as significantly enriched. The top pathway enriched was the

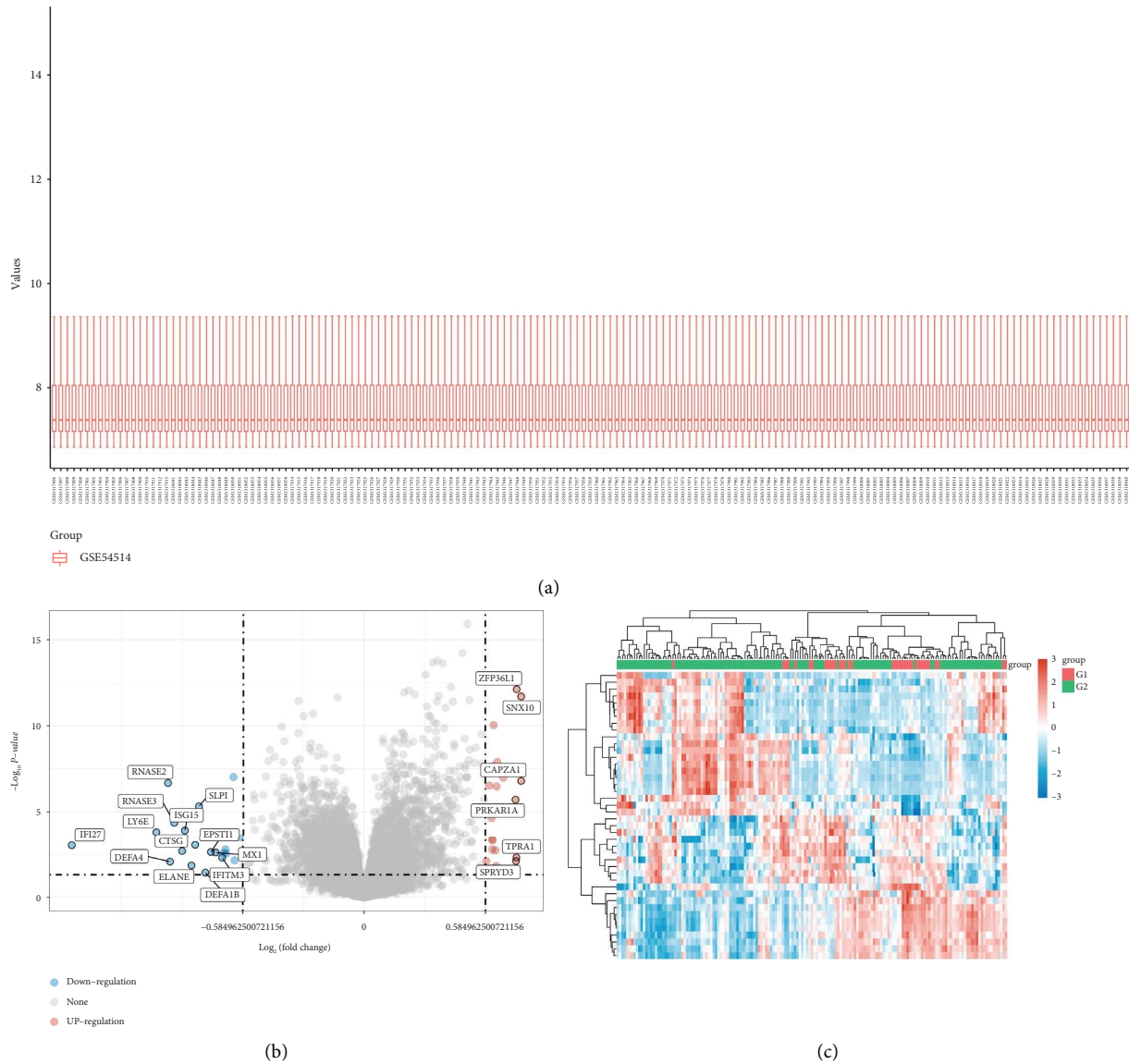


FIGURE 1: (a) The map showed the samples of patients in the sepsis cohort and normal people; (b) the volcano map showed the differentially expressed genes between the normal group and patients in the sepsis cohort; (c) the heatmap showed the expression level of key genes in GSE54514.

“ribosome” pathway (KEGG\_RIBOSOME) with an enrichment score of approximately 0.762. Following closely was the pathway known as “graft-versus-host disease” (KEGG\_GRAFT\_VERSUS\_HOST\_DISEASE), which had an enrichment score of approximately 0.782. Additional significant pathways are “cell adhesion molecules (CAMs)” (KEGG\_CELL\_ADHESION\_MOLECULES\_CAMS) and “type I diabetes mellitus” (KEGG\_TYPE\_I\_DIABETES\_MELLITUS) exhibiting enrichment scores of 0.598 and 0.763 correspondingly. The findings indicate that the gene CCL5 is closely linked to multiple biological pathways, potentially influencing cellular processes such as ribosome functionality, immune response in graft-versus-host disease, cell adhesion, and type I diabetes mellitus (Figure 5(a)). During our GSEA examination of CD274, the pathway “ribosome” (KEGG\_RIBOSOME) emerged as the most

highly enriched, displaying an adjusted  $p$  value close to  $1.81 \times 10^{-8}$ . Additional pathways that are pertinent include “autoimmune thyroid disorder” (KEGG\_AUTOIMMUNE\_THYROID\_DISORDER), “type 1 diabetes” (KEGG\_TYPE\_1\_DIABETES), and “graft-versus-host disorder” (KEGG\_GRAFT\_VERSUS\_HOST\_DISORDER). These findings suggest potential biological processes linked with CD274 expression (Figure 5(b)). The GSEA analysis focused on CD3E revealed that the “ribosome” pathway (KEGG\_RIBOSOME) showed the highest level of enrichment, with an adjusted  $p$  value less than 0.05. Additional notable pathways include “cell adhesion molecules (CAMs)” (KEGG\_CELL\_ADHESION\_MOLECULES\_CAMS) and “autoimmune thyroid disease” (KEGG\_AUTOIMMUNE\_THYROID\_DISEASE). The results emphasize possible biological mechanisms and routes linked to the

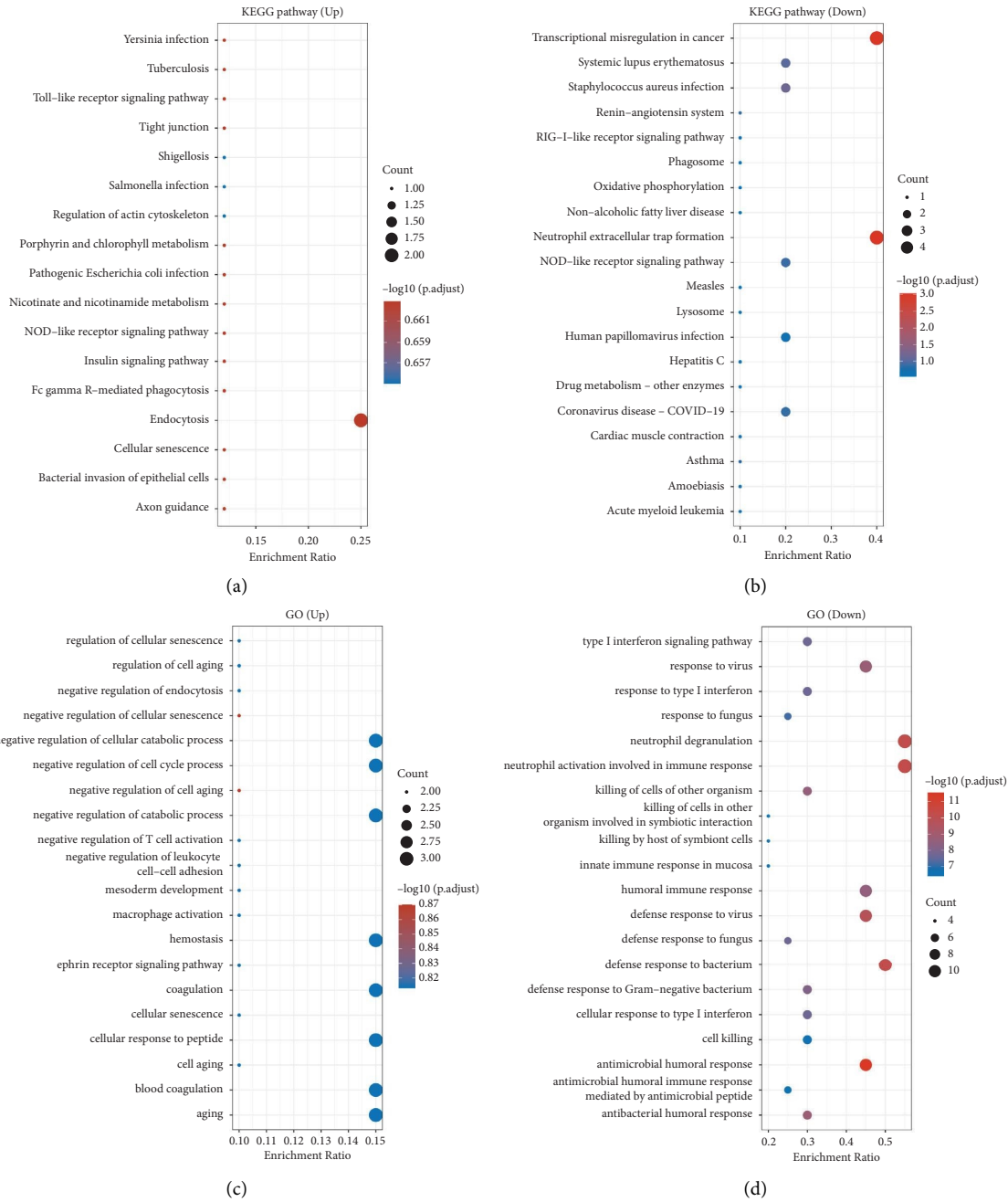


FIGURE 2: (a) The KEGG enrichment analysis of the upregulation genes; (b) the KEGG enrichment of downregulation genes; (c) the GO enrichment analysis of the upregulation genes; (d) the GO enrichment analysis of the downregulation genes.

manifestation of CD3E (Figure 5(c)). In CD8A's GSEA analysis, the pathway "ribosome" (KEGG\_RIBOSOME) emerged as the highly enriched, showing an adjusted  $p$  value of less than 0.05. Additional notable pathways consist of "autoimmune thyroid disease" (KEGG\_AUTOIMMUNE\_THYROID\_DISEASE) and "type I diabetes mellitus" (KEGG\_TYPE\_I\_DIABETES\_MELLITUS). These data point to potential biological interactions and processes associated with CD8A expression (Figure 5(d)).

#### 4. Discussion

Sepsis, a life-threatening condition often triggered by an infection, has long been a challenging area of study due to its multifaceted nature and the myriad of molecular and cellular processes involved. The analysis of the GSE54514 dataset has yielded new understandings regarding the gene expression profiles and pathways linked to sepsis and its interaction with the immune system.

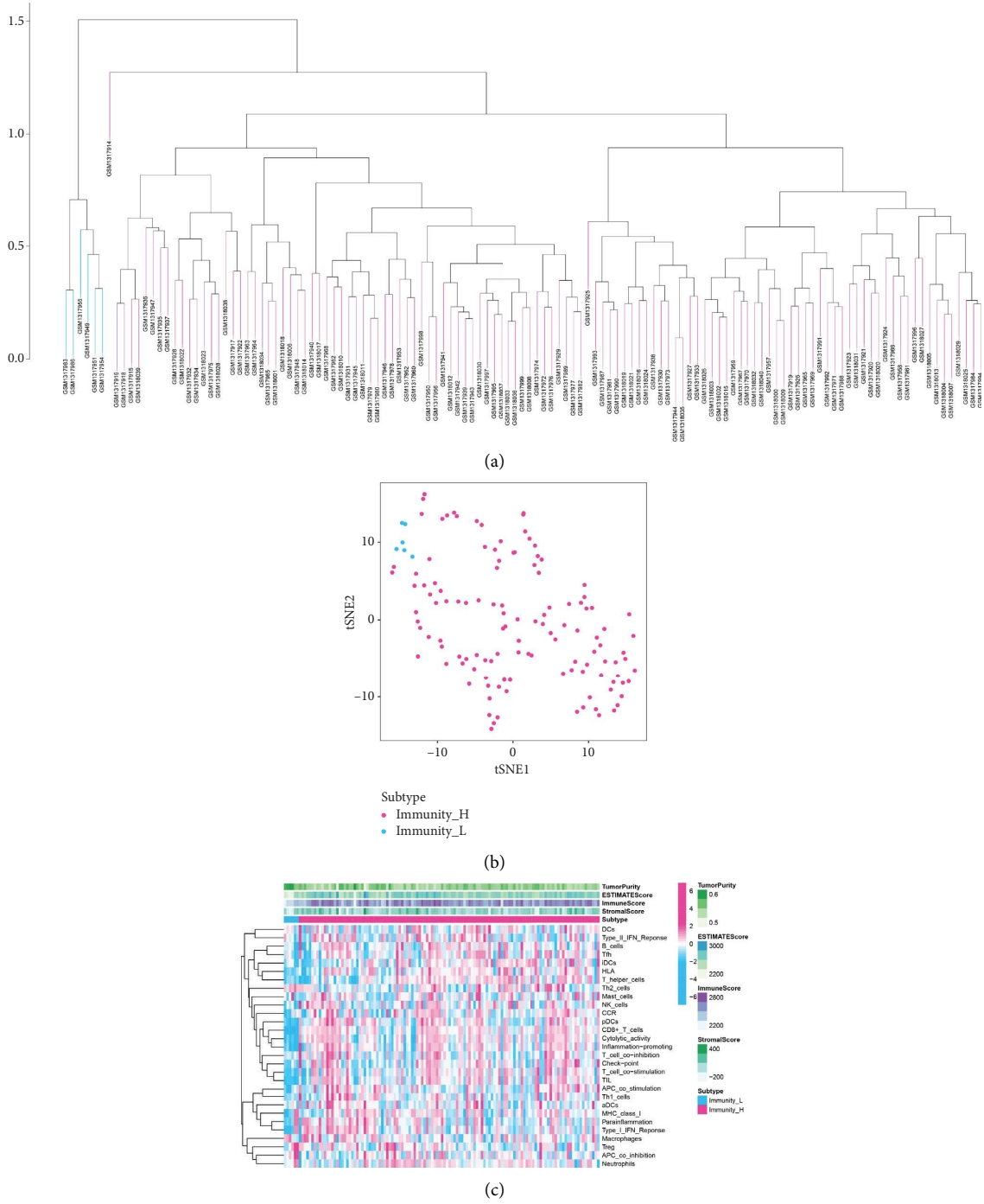
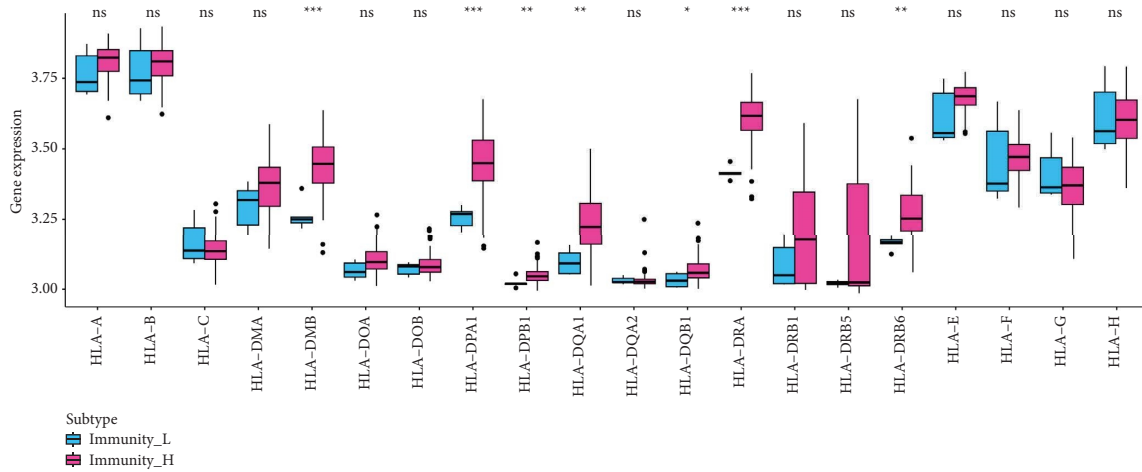
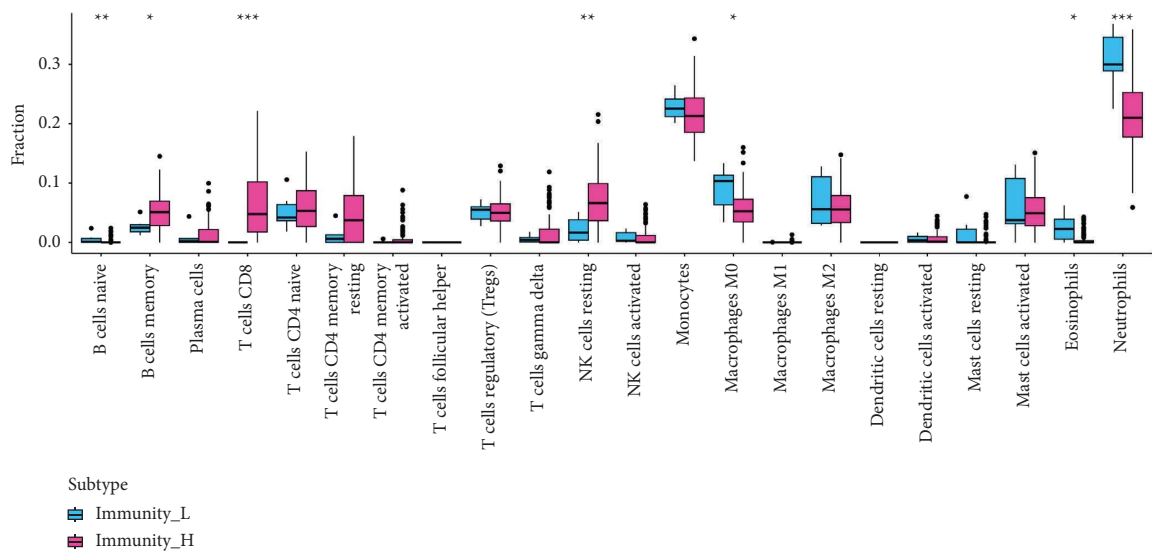


FIGURE 3: Continued.



(d)

FIGURE 3: (a) The ssGSEA analysis showed the immune-related score in the sepsis cohort; (b) the Tsne analysis of the patients in the sepsis cohort; (c) the heatmap showed the immune-related scores in the sepsis cohort; (d) the boxplot showed the expression level of HLA-related genes between low- and high-immune-related groups.



(a)

FIGURE 4: Continued.

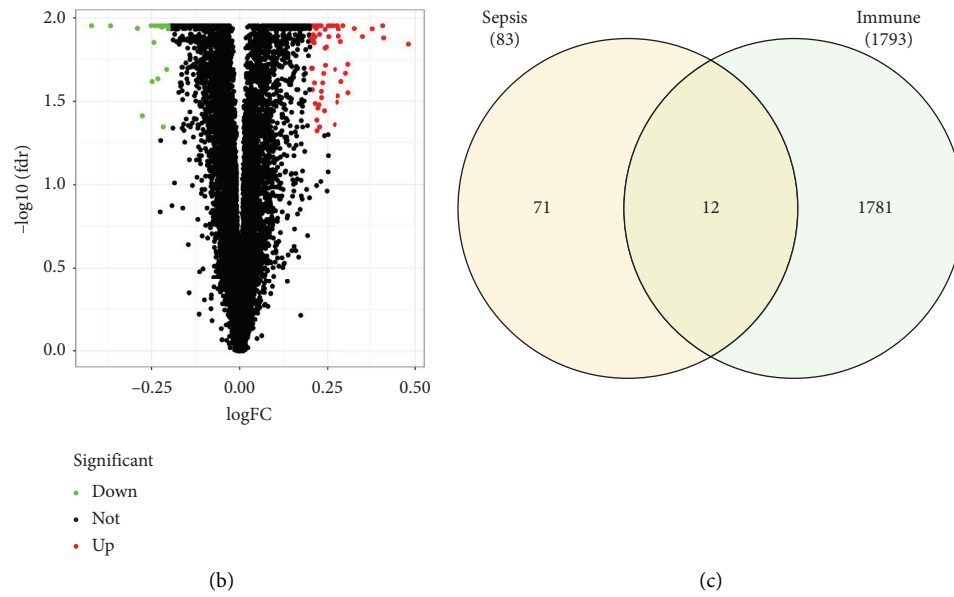


FIGURE 4: (a) The immune cell infiltration level between low- and high-immune-related groups; (b) the volcano map showed the differentially expressed genes between low- and high-immune related groups; (c) the Venn diagram showed the key immune-related genes in the sepsis cohort.

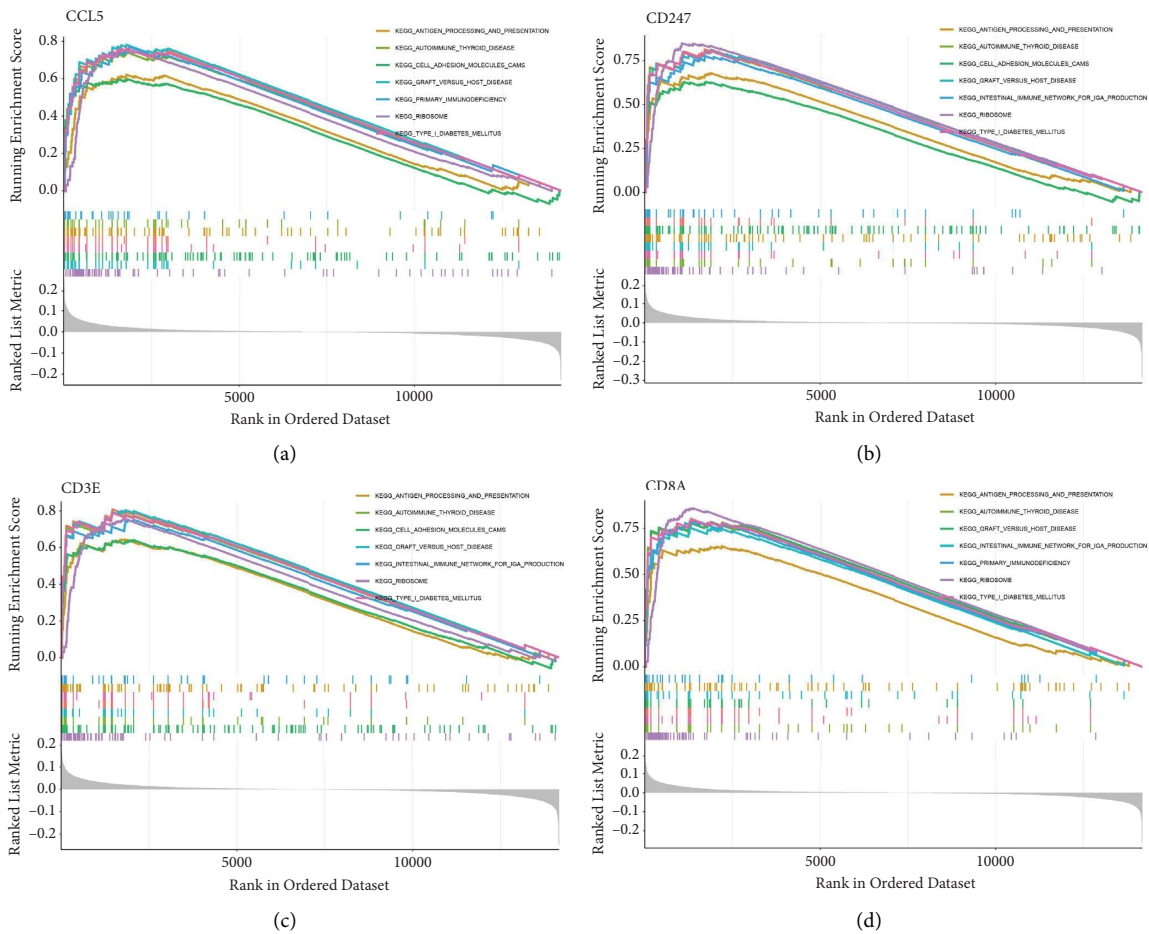


FIGURE 5: (a) The GSEA analysis of CCL5 in KEGG terms; (b) the GSEA analysis of CD3E in KEGG terms; (c) the GSEA analysis of CD8A in KEGG terms; (d) the GSEA analysis of CD247 in KEGG terms.



A significant discovery in our research was the recognition of genes that were expressed differently in healthy individuals compared to those with sepsis. During sepsis, there are 42 genes that exhibit significant molecular changes, with an equal distribution of upregulated and down-regulated expressions. These genes have the potential to function as biomarkers for the early identification, prognosis, or even treatment targets, but additional validation studies are necessary to verify these functions.

The analysis of pathway enrichment offered a more profound comprehension of the disrupted biological processes in sepsis. The pathway of toll-like receptors, which is essential for the body's natural defense system, is one of the highly enriched pathways, further supporting the notion that sepsis interferes with the initial barrier against harmful microorganisms. Similarly, the participation in the control of actin cytoskeleton implies potential alterations in cellular structure, potentially associated with the movement of immune cells and engulfment of particles. The enhancement of the NOD-like and RIG-1-like receptor signaling pathways underscores the significant influence of sepsis on the recognition mechanisms of intracellular pathogens. These findings resonate well with previous studies that have also highlighted the dysregulation of innate immune pathways in sepsis.

The ssGSEA examination was especially illuminating in providing insight into the correlation between sepsis and the dynamics of immune cells. It is interesting to observe that the manifestation of specific HLA-associated genes, crucial for presenting antigens, exhibited a strong correlation with an elevated immune-related atmosphere. This implies that, in the context of sepsis, there could be an intensified endeavor from the immune system to showcase antigens and initiate a protective reaction despite the possibility of an imbalanced or potentially detrimental immune response. The intricate equilibrium of immune cell populations during sepsis is further demonstrated by the notable associations among specific immune cells, including memory B cells, inexperienced B cells, and CD8 T cells, with the immune-related score. These findings align with the concept that sepsis can cause both hyperinflammation and immune paralysis.

In addition, our examination emphasized particular genes, such as CCL5, CD274, CD3E, and CD8A, along with the pathways they are linked to. The frequent enrichment of the "ribosome" pathway across these genes is intriguing. Ribosomes, while primarily recognized for their role in protein synthesis, have been recently implicated in immune responses, especially in the context of viral infections. The results of our study could indicate a wider function of ribosomes in sepsis, potentially associated with the swift production of proteins involved in the immune response. The repeated emergence of pathways related to autoimmune diseases in the GSEA analysis, such as "autoimmune thyroid disease" and "type I diabetes mellitus," might hint at the autoinflammatory nature of sepsis. It raises the question of whether sepsis, in some patients, triggers or exacerbates underlying autoimmune tendencies.

To summarize, our thorough examination has yielded valuable understandings regarding the dynamics of gene expression and disruptions in pathways during sepsis. Further investigation can be conducted to explore the complex relationship between sepsis and the immune system, particularly the intricate changes in immune cell populations and their functionalities. Our findings pave the way for future studies focusing on therapeutic interventions targeting these identified pathways and genes. Understanding the molecular intricacies of sepsis is crucial not just for better diagnostics and prognostics but also for developing more effective and targeted treatments for this complex condition.

## Data Availability

The data used to support the findings of this study are included within the article.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

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