Screening of Relevant Genes and Signalling Pathways Affecting Adult Urosepsis: A Bioinformatic Analysis

Xu Xi Shi[1], Lu Rong Rong[2], Huang Zhong Wei[1]*

Purpose: The cellular and molecular pathophysiology of urosepsis, a condition caused by a urinary tract infection spreading to the bloodstream, involves complex epigenetic behavior. The objective of this study was to identify relevant genes and signaling pathways in adult urosepsis through a bioinformatic analysis of differentially expressed genes (DEGs).

Materials and Methods: In this in silico study, the GSE69528 dataset, containing 138 total RNA blood samples from patients with sepsis and uninfected controls, was obtained from the Gene Expression Omnibus (GEO) database. Microarray data were analyzed using GEO2R tools and R software. DEGs were identified using a fold change (FC) cutoff of > 1.5 or < 0.67 and a significance level of $p < 0.05$. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were used to determine the enriched pathways of DEGs before constructing protein-protein interaction (PPI) networks with STRING and Cytoscape.

Results: A total of 108 DEGs were identified, comprising 67 upregulated and 41 downregulated genes. GO and KEGG analyses revealed that these DEGs were significantly enriched in pathways such as the complement and coagulation cascade, neutrophil degranulation, negative regulation of interferon-gamma response, T-cell activation, and granulocyte differentiation. The PPI network analysis identified 67 nodes with 110 interactions, from which CEACAM8, MPO, and RETN were identified as hub genes. Overexpression of CEACAM8 and MPO and suppression of RETN may be associated with a better disease prognosis.

Conclusion: The identified hub genes—CEACAM8, MPO, and RETN—are predicted to be significant biomarkers in the prognosis and progression of sepsis. These genes could be targeted for the discovery of new therapeutic drugs for treating and managing urosepsis.

Keywords: benign prostatic hyperplasia; holmium laser; prostatic adenoma; transurethral prostate resection

INTRODUCTION

Sepsis is a life-threatening medical condition characterized by a systemic inflammatory response to infection, leading to organ dysfunction.[1,2] Urosepsis is a type of sepsis caused by a bacterial infection of the urinary tract, typically associated with enterobacteria such as *E. coli*, enterococci, and *Klebsiella* spp.[1] The condition has a high mortality rate, with approximately 700 cases per 100,000 people worldwide, and requires early diagnosis and treatment to increase survival rates.[2]

The pathophysiology of sepsis involves a complex interplay between microbial-derived pathogen-associated molecular patterns (PAMPs) and endogenous damage-associated molecular patterns (DAMPs).[3] PAMPs, such as lipopolysaccharides and peptidoglycans, are recognized by the innate immune system, triggering a signaling cascade that activates proinflammatory responses.[3] DAMPs, including high-mobility group box 1 (HMGB1) and heat shock proteins, are released from damaged cells and also activate the innate immune system, leading to the recruitment of immune cells and the production of proinflammatory cytokines.[3] The clinical manifestations of sepsis include hyperlactatemia, hypotension, and organ dysfunction, with a mortality rate that can exceed 50% in intensive care units.[4]

The Human Genome Project, completed in 2003, provided a comprehensive map of the human genome, enabling researchers to identify genetic variations associated with an increased risk of complex diseases.[5] The subsequent development of high-throughput sequencing technologies has further accelerated the discovery of genetic variants and their functional characterization.[6] However, the relationship between genetic variation and disease is complex. Epigenetic modifications, gene-environment interactions, and the influence of non-coding RNAs on gene expression all play critical roles in shaping the phenotypic outcomes of genetic variation.[7-9]

During sepsis, the host's immune response is characterized by a delicate balance between proinflammatory and anti-inflammatory responses.[10] Epigenetic mechanisms can either promote or suppress the expression of genes involved in the immune response, thereby influencing the severity of sepsis.[11] Epigenetic biomarkers, such as DNA methylation patterns and miRNA ex-

[1]Affiliated Hospital of Nantong University, Emergency Department, Nantong, 226000, Jiangsu, China.
[2]Jiangsu Rudong County People's Hospital, Pharmacy Department, Nantong, 226400, Jiangsu, China.
*Correspondence: Affiliated Hospital of Nantong University, Emergency Department Nantong, 226000, Jiangsu, China.
E-mail: medicalpaper203@gmail.com.
Received August 2023 & Accepted September 2025

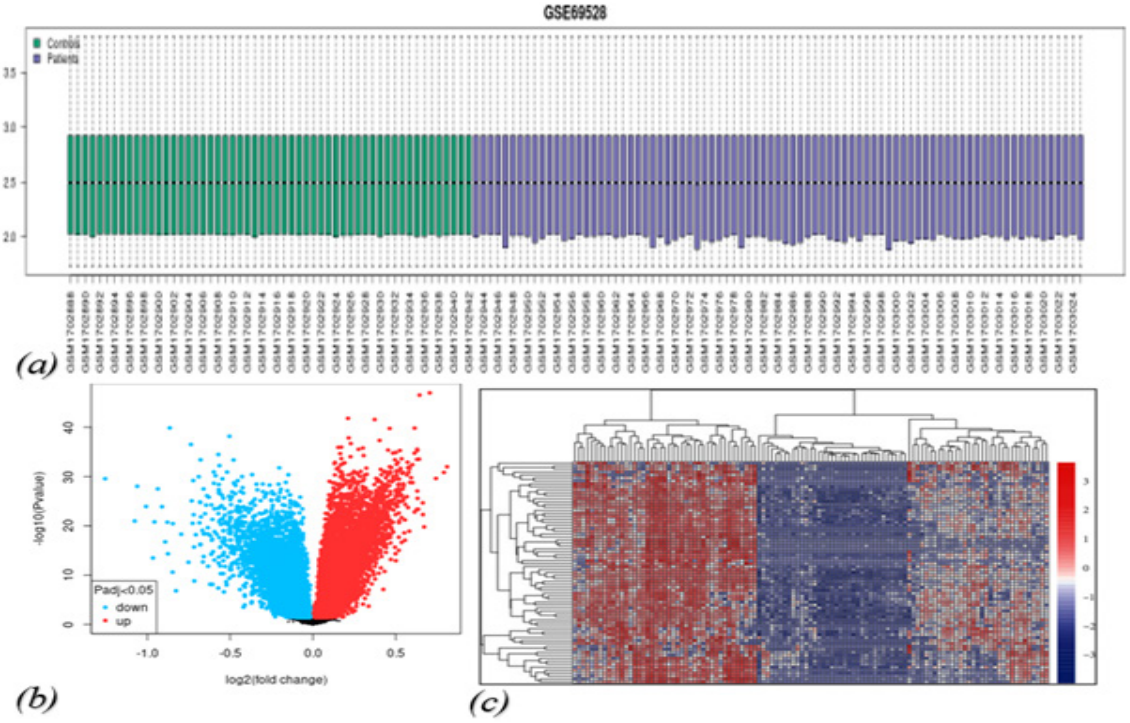

Figure 1. (a) Box-and-whisker plot of normalized gene expression profiles; **(b)** volcano plot of upregulated and downregulated genes; **(c)** heatmap of DEGs.

pression profiles, may help predict sepsis severity and guide treatment decisions.[12] Specific DNA methylation patterns have been associated with sepsis severity and outcome.[13] For example, methylation of the *IL-10* gene promoter has been linked to reduced IL-10 expression and increased inflammation in sepsis,[14] as has methylation of the *TNF-α* gene promoter[15] and the *SOCS3* gene.[16]

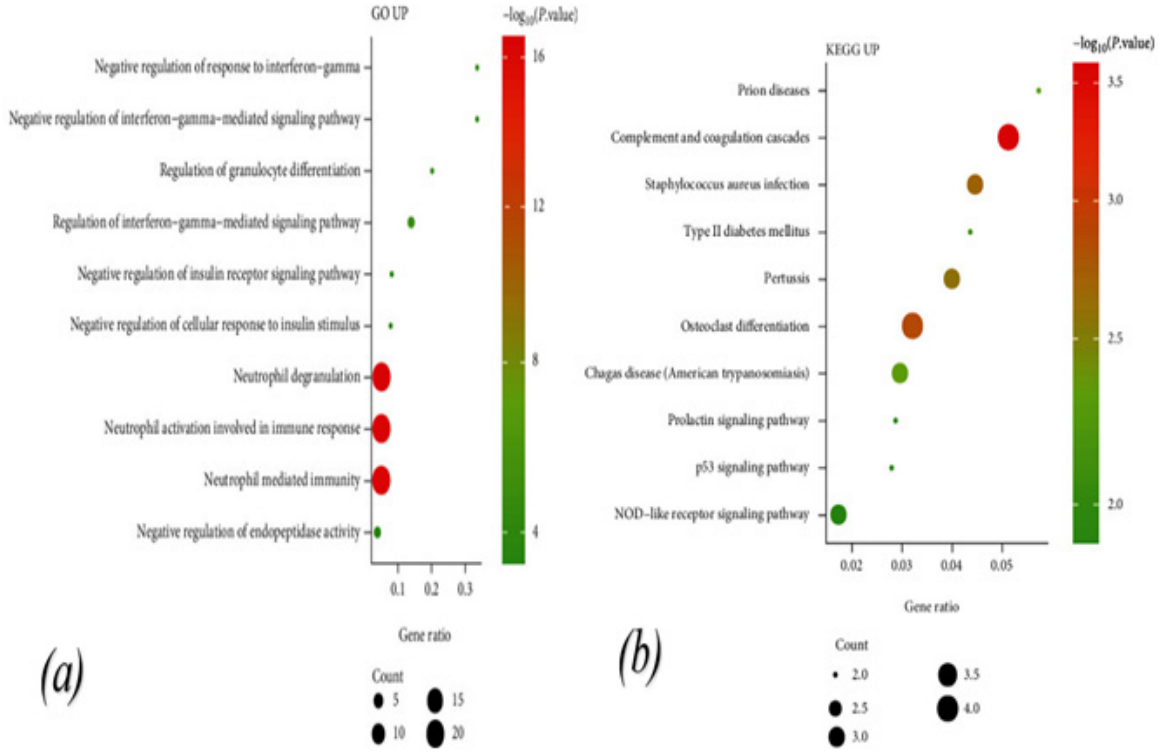

Figure 2. (a) Biological processes of upregulated genes; **(b)** KEGG pathways of upregulated genes.

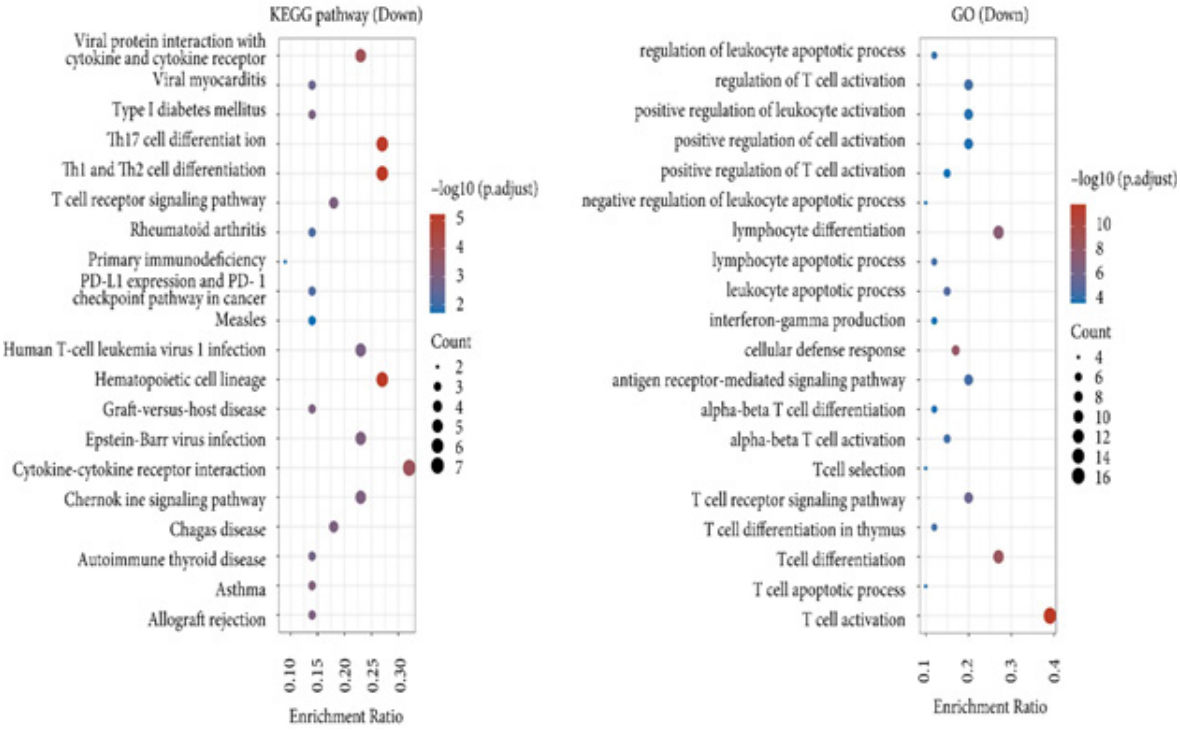

Figure 3. KEGG and GO pathways of downregulated genes. The enrichment ratio is shown at the bottom, the p-value is shown by color, and the size of the circles indicates the number of genes.

Recent advances in high-throughput technologies have enabled the identification of differentially expressed genes (DEGs) that contribute to the pathogenesis of sepsis. Studies have consistently shown that genes involved in the innate immune response, such as Toll-like receptor 4 (TLR4),(17) CD14,[18] and lipopolysaccharide-binding protein (LBP),[19] are upregulated in septic patients. Conversely, genes involved in the regulation of inflammation, such as interleukin-10 (IL-10) [20] and tumor necrosis factor-alpha-induced protein 3 (TNFAIP3),[21] are often downregulated, suggesting a disrupted balance between pro- and anti-inflammatory responses. The analysis of DEGs can provide insights into the biological mechanisms underlying sepsis and help identify potential novel diagnostic biomarkers. Therefore, the objective of this study was to identify relevant genes and signaling pathways affecting adult urosepsis by performing a bioinformatic analysis of DEGs.

MATERIALS AND METHODS

Extraction of Microarray Data and Processing

This was an in silico study. To identify relevant gene expression datasets, we searched the Gene Expression Omnibus (GEO) database using the keywords: (Sepsis OR Urosepsis) AND (Gene Expression Profiling OR Microarray Analysis OR RNA sequencing). We limited our search to datasets from *Homo sapiens* and filtered for "Expression profiling by high throughput sequencing" or "Transcription profiling by array." This search identified the GSE69528 dataset, which comprised 138 whole blood RNA samples from patients with septic conditions (n=83) and uninfected controls (n=55). The limma R package was used to perform background correction, quartile normalization, and summarization of probe-level data. The mean of the probes was taken as the representative measure of gene expression levels.

Identification of DEGs

The raw gene expression dataset GSE69528 was analyzed using the GEO2R tool and the Limma and Bioconductor packages in R software. Volcano plots, box plots, and adjusted p-values were generated to provide a comprehensive overview of the dataset. A statistical threshold of $p < 0.05$ and a fold change (FC) cutoff of either $FC > 1.5$ or $FC < 0.67$ were used to identify DEGs. A heatmap was constructed to visualize the expression levels of up- and downregulated genes, using a hierarchical clustering approach with the Euclidean distance metric.

Pathway and Gene Ontology of DEGs

Gene Ontology (GO) analysis was performed using the ToppGene functional annotation tool to classify genes into molecular functions, cellular components, and biological processes. Pathway enrichment analysis was conducted based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database to discover the biological functions and mechanisms of the identified genes.

Protein-Protein Interaction (PPI) Networks

PPI networks were analyzed using the Search Tool for the Retrieval of Interacting Genes (STRING) database. An interaction score greater than 0.4 was considered significant. The Cytoscape software was used to visualize the networks, and the MCODE plugin was used to extract hub genes based on node degree.

Ethical Approval

This was a secondary data analysis and did not require ethical approval.

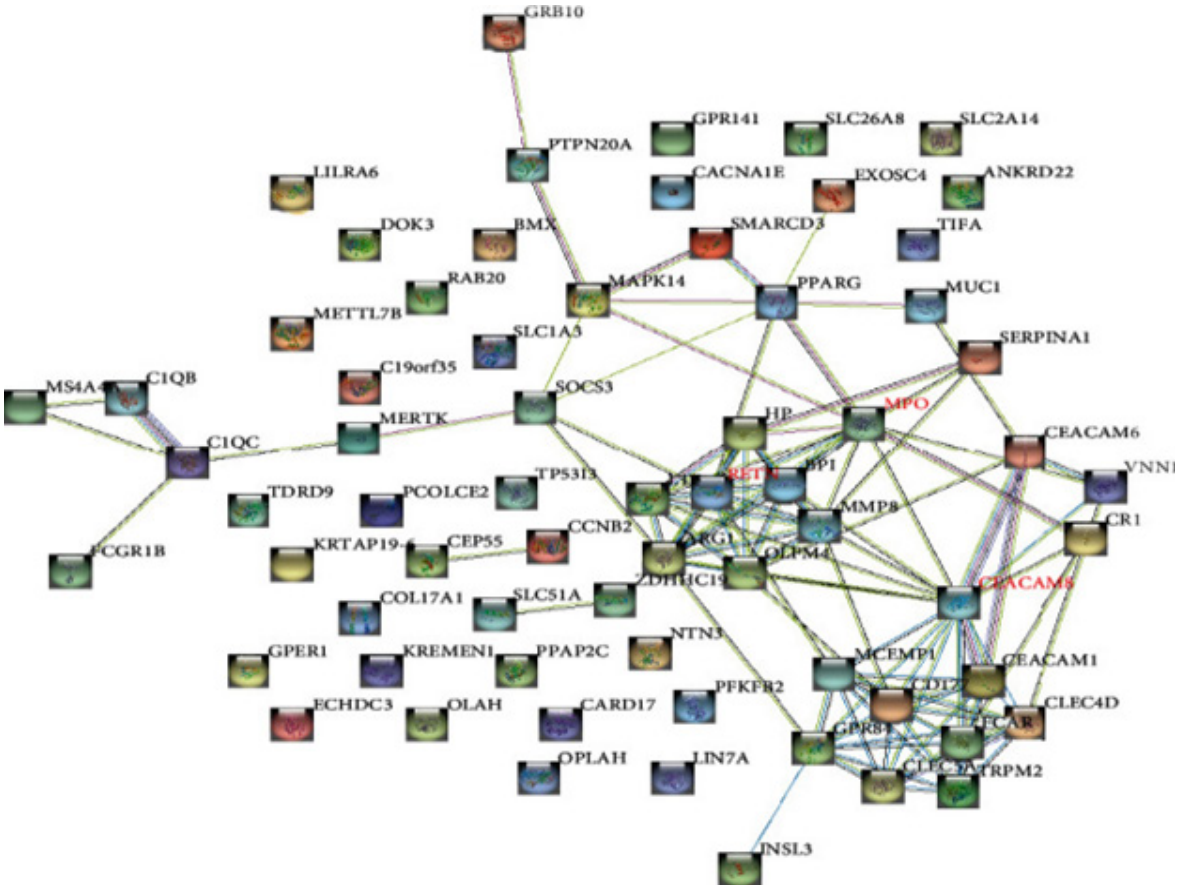

Figure 4. PPI network showing the association of proteins from upregulated genes.

RESULTS

Data and Identification of DEGs

The GSE69528 dataset contained gene expression profiles from 55 healthy controls and 83 septic patients. After normalization and log2 transformation of the raw data, the analysis identified 108 DEGs, consisting of 67 upregulated and 41 downregulated genes. The effectiveness of normalization was confirmed by boxplots, which showed a consistent distribution of gene expression values across samples (**Figure 1a**). Figure 1b presents a volcano plot illustrating the DEGs, with upregulated genes shown on the right and downregulated genes on the left. In Figure 1c, a heatmap clustering the DEGs shows a clear distinction between the control and patient groups, with upregulated genes in red and downregulated genes in blue.

GO and Pathway Enrichment Analysis

Enrichment analysis revealed that the upregulated DEGs were significantly enriched in biological processes related to the regulation of granulocyte differentiation and the negative regulation of interferon-gamma (IFN-γ) responses (**Figure 2a**). KEGG pathway analysis indicated that upregulated genes were significantly enriched in pathways associated with *Staphylococcus aureus* infections, complement and coagulation cascades, and prion diseases (**Figure 2b**). In contrast, the downregulated genes were primarily enriched in cytokine-cytokine receptor interactions (KEGG) and T-cell activation (GO) (**Figure 3**).

PPI Networks

A PPI network of upregulated genes was constructed using the STRING database (**Figure 4**). The resulting network consisted of 67 nodes and 110 edges. Degree analysis in Cytoscape identified several hub genes with a high number of connections, suggesting their potential importance in sepsis. Notably, CEACAM8 (degree = 17), MPO (degree = 12), and RETN were identified as key hub genes.

DISCUSSION

Our study aimed to identify relevant genes and signaling pathways in adult urosepsis through a bioinformatic analysis. We identified 108 DEGs (67 upregulated, 41 downregulated) that were significantly enriched in immune-related pathways, including the complement and coagulation cascade, neutrophil degranulation, and negative regulation of interferon-gamma. These findings are consistent with other studies that have identified immune-related pathways as being highly enriched in sepsis. For instance, a study by Choi et al. used a similar bioinformatic approach and found that key upregulated genes were involved in cell cycle regulation and immune responses.[22]

The differences in the number and types of DEGs identified across various studies can be attributed to differences in the datasets used, the statistical cutoffs for DEG identification, and the specific focus of the research. For example, some studies used weighted gene co-expression network analysis (WGCNA)(23) or machine learning algorithms[24] to identify hub genes,

whereas our study relied on standard differential expression analysis followed by pathway enrichment. Other studies have also analyzed the GSE69528 dataset but reported slightly different hub genes, likely due to variations in analytical methods.[25-28] The identification of overlapping genes across multiple studies, such as PTEN and HIST2H2BE, suggests that these genes may be critical in the pathogenesis of sepsis.[28] However, further validation and replication studies are necessary to confirm these findings and establish their clinical utility.

In our study, CEACAM8, RETN, and MPO were identified as hub genes. The results suggest that overexpression of CEACAM8 and MPO, along with the suppression of RETN, might be associated with a better disease prognosis. The CEACAM8 gene is known to be upregulated in sepsis patients, and its upregulation is associated with NETosis and sepsis progression.[29] The exact mechanism of CEACAM8 upregulation is not fully understood but may be related to the presence of extracellular chromatin.[30] The clinical implications of CEACAM8 upregulation are significant, and further studies are needed to explore its potential as a diagnostic and prognostic biomarker for sepsis.[31]

CONCLUSIONS

This study identified three key genes—CEACAM8, RETN, and MPO—that may play a crucial role in the diagnosis and prognosis of sepsis. The results suggest that overexpression of CEACAM8 and MPO may be associated with a better prognosis, while suppression of RETN may also be beneficial. CEACAM8 is involved in the recruitment and activation of neutrophils, and MPO enhances their antimicrobial activity. Conversely, RETN appears to inhibit neutrophil activity, which could lead to immunosuppression and increased severity of urosepsis. Analysis of key signaling pathways revealed that the negative regulation of interferon-gamma, granulocyte differentiation, and the complement and coagulation cascades are significant in sepsis. Targeting these genes and pathways may offer potential therapeutic approaches for managing urosepsis.

SUMMARY

Computer analysis identified three key genes (CEACAM8, MPO, RETN) in urosepsis, a severe blood infection from a UTI. These genes are potential biomarkers for prognosis and targets for new drugs to help manage this life-threatening condition.

ACKNOWLEDGEMENTS

The authors received no specific funding for this work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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