ORIGINAL RESEARCH

Identification of Key Inflammation-related Genes as Potential Diagnostic Biomarkers of Sepsis

Peng Guo, MM; Runze Wang, MM; Jie Shen, PhD; Lin Zhang, PhD; Weichun Mo, MM

ABSTRACT

Context • Sepsis is one of the leading causes of mortality for patients with severe infections who had been admitted to intensive care units (ICUs). Early diagnosis, accurate treatment, and management of sepsis remain extremely difficult in clinical settings, due to a lack of early biomarkers and diverse clinical manifestations.

Objective • The study intended to identify the key genes and pathways associated with inflammation in sepsis—using microarray technology combined with bioinformatics and key inflammation-related genes (IRGs)—to perform an enrichment analysis and evaluate the value of those genes for the diagnosis and evaluation of prognosis for patients with sepsis.

Design • The research team performed a genetic analysis.

Setting • The study took place at the Center for Emergency and Critical Medicine at Jinshan Hospital of Fudan University in Jinshan District, Shanghai, China.

Groups • The research team created two groups, the sepsis group, individuals with sepsis, and the control group, individuals without sepsis, using data for those groups from five microarray datasets obtained from the Gene Expression Omnibus (GEO) database.

Outcome Measures • The research team: (1) downloaded the GSE57065, GSE28750, GSE9692, GSE13904, and GSE54514 datasets from the Gene Expression Omnibus (GEO) database for analysis; (2) analyzed the GSE57065, GSE28750, and GSE9692 datasets to detect the differentially expressed genes (DEGs) in the sepsis and control groups; (3) used Venn diagrams to obtain the intersection of DEGs and inflammation-related genes (IRGs); (4) mapped the protein-protein interaction (PPI) network using the Search Tool for Retrieval of Interacting Genes (STRING) database; (5) detected the hub genes using Cytoscape and cytoHubba; (6) performed an enrichment analysis of hub IRGs using Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG); (7) validated the expression of hub IRGs in sepsis using the GSE13904 dataset; and (8) performed a survival analysis in sepsis using the GSE54514 dataset to explore the prognostic value of the hub IRGs.

Results • The research team: (1) identified 104 upregulated DEGs and 4 downregulated DEGs; (2) after defining the intersection of DEGs and IRGs, detected nine differentially expressed IRGs (DEIRGs); and (3) identified five IRGs—haptoglobin (HP), high affinity immunoglobulin gamma Fc receptor I (FCGR1A), cluster of differentiation 163 (CD163), complement C3a receptor 1 human (C3AR1), C-type lectin domain containing 5A (CLEC5A)—that overlapped DEIRGs. The GO and KEGG pathway analyses showed that the hub IRGs became enriched during acute-phase response, acute inflammatory response, specific granule, specific granule membrane, endocytic vesicle membrane, tertiary granule, immunoglobulin G (IgG) binding, complement receptor activity, Ig binding, scavenger receptor activity, and scaffold protein binding. The DEGs also played a significant role in Staphylococcus aureus (S. aureus) infection. The ROC curves showed that HP (AUC: 0.956, 95% CI: 0.924–0.988); FCGR1A (AUC: 0.895,95% CI: 0.827–0.963); CD163 (AUC: 0.838, 95% CI: 0.774–0.901); C3AR1 (AUC: 0.953, 95% CI: 0.913–0.993); and CLEC5A (AUC: 0.951, 95% CI: 0 920–0 981) had meaningful diagnostic value for sepsis. Survival analysis showed that the sepsis and control groups had significant differences in HP ($P = .043$) and CLEC5A ($P < .001$).

Conclusions • HP, FCGR1A, CD163, C3AR1, and CLEC5A have value for clinical application. Clinicians can use them as diagnostic biomarkers, and they provide research direction for treatment targets for sepsis. (*Altern Ther Health Med.* 2023;29(5):24-31).

Peng Guo, MM, Resident Doctor; **Runze Wang,** MM, Attending Doctor; **Jie Shen,** PhD, Chief Physician; **Lin Zhang,** PhD, Associate Chief Physician; **Weichun Mo,** MM, Associate Chief Physician; Center for Emergency and Critical Medicine, Jinshan Hospital of Fudan University, Jinshan District, Shanghai, China.

Corresponding author: Weichun Mo, MM
E-mail: 18930815698@163.com

Sepsis refers to a group of clinical disorders that are characterized by systemic organ failure due to an uncontrolled host reaction toward an infection.[1-3] It's one of the leading causes of mortality for patients with severe infections who have been admitted to intensive care units (ICUs).

Currently, sepsis still has an impressively increasing incidence, high mortality, and ever-rising costs, and it presents challenges for clinicians.[4,5] Current treatment focuses mainly on early fluid resuscitation and anti-infective empirical

treatment; timely diagnosis and precise treatment could significantly improve the prognosis of patients with sepsis.[6]

Although early diagnosis of sepsis is associated with patients' improved prognosis, so far accurate biomarkers to diagnose sepsis are lacking. Researchers have demonstrated that both procalcitonin (PCT) and soluble triggering receptor expressed on myeloid cell-1 (sTREM-1) can be useful in its early diagnosis but haven't yet proven their effectiveness for sepsis in clinical settings.[7,8]

Despite decades of research and exploration, early diagnosis, accurate treatment, and management of sepsis remain extremely difficult in clinical settings, due to a lack of early biomarkers and diverse clinical manifestations.[9,10]

The pathogenesis of sepsis involves multiple factors that induce a dysregulated immune response and an inflammatory storm, but the immune stage of sepsis, which involves immune pathways and multiple biological processes, is still not completely clear.

Inflammation-related Genes

C3AR1 and FCGR1A. Naber found that *S. aureus* is one of the most common blood isolates during sepsis.[11] In sepsis that *Staphylococcus aureus* (*S. aureus*) causes, a number of factors are expressed that interfere with the efficiency of neutrophils, which are a major source of inflammatory cells that respond to infection, and of macrophages, by suppressing complementary activation, neutrophil chemotaxis, neutrophil lysis, or immune evasion.[12] Furthermore, *S. aureus* can exacerbate symptoms in infected individuals by boosting immunosuppression and impairing the normal humoral immune response.[13]

Complement C3a receptor 1 (C3AR1) and Fc gamma receptor 1A (FCGR1A) are key regulators of inflammation. In the pathway of *S. aureus* infection, they play an important role in its immune evasion and may be correlated with the inhibition of chemotaxis and phagocyte activation.[14]

Brennan et al found that C3AR1 can antagonize neutrophil chemotactic signals, inversely controlling neutrophil mobility.[15] The majority of immune cells express FCGR1A as one of Fc-gamma receptors (FcγRs). This involves monocytes, NK cells, B cells, eosinophils, basophils, dendritic cells, platelets, macrophages, and even some subpopulations of T cells.[16,17]

Antibody-mediated phagocytosis involves expression of FcγRs on monocytes, dendritic cells (DCs), macrophages, and neutrophils.[16] A protein that the gene FCGR1A encodes has a vital role in the immune reaction. Its surface expression can rapidly increase in response to infection, mediated by bacterial lipopolysaccharides (LPS), interleukin-12 (IL-12), interferon (IFN), and granulocyte colony-stimulating factor (G-CSF).

FCGR1A might be a good diagnostic indicator of infectious diseases.[18] Yang et al found that C3AR1 overexpression was associated with poor prognosis and enhanced infiltration of tumor-immune adenocarcinomas of the stomach.[19] Wu et al found a significant correlation between FCGR1A genotypes and sarcoidosis vulnerability and intensity.[20]

CD163 and CLEC5A. Cluster of differentiation 163 (CD163) and C-type lectin domain containing 5A (CLEC5A) also can significantly affect inflammatory responses and regulation of macrophage function.

CD163 has a critical role in the defense against contagious diseases. Expression of CD163 occurs only through macrophages at a high level and only through monocytes at a low level. Although CD163 is commonly thought to be an M2 marker, it appears that only a small percentage of M2s are CD163+.[21]

In coronary heart disease, Guo et al found that CD163 expression was upregulated and linked to the development of atherosclerosis and probable complications.[22] Researchers have also found upregulated CD163 in some malignant diseases, such as classical Hodgkin lymphoma[23] and diffuse large B-cell lymphoma.[24]

Chen et al found CLEC5A regulates pro-inflammatory cytokine release from macrophages and is a confirmed key factor in the processing of Japanese encephalitis virus (JEV)-induced neuroinflammation.[25] Chen et al found that the percentage of CLEC5A-expressing monocytes and their expression levels dramatically increased in autoinflammatory diseases, such as rheumatoid arthritis (RA) and adult-onset Still disease (AOSD).[26] In Tosiek et al's study, the mixed response of pro- and anti-inflammatory cytokines to an unclear immune reaction and the regulation of the expression of the myeloid cell receptor in two opposing directions were both indicative of the immune response in sterile settings that a chosen CLEC5A agonist produced.[27]

Chen et al found that CLEC5A, through the stimulation of the activities of macrophages, neutrophils, and T cell-mediated effectors, can be a major pathogen recognition receptor (PRR) in immunological responses to bacteria, including *L. monocytogenes* and *S. aureus*.[28] Sung et al found that CLEC5A can promote thromboinflammation, exacerbating acute respiratory distress syndrome (ARDS) risk for coronavirus disease 2019 (COVID-19) patients, which highlights the importance of CLEC5A and toll-like receptor 2 (TLR2) as therapeutic targets.[29]

Haptoglobin (HP). HP is a plasma hemoglobin-binding protein that aids in the release of hemoglobin from red blood cells following either intravascular or extravascular hemolysis. Patients with sepsis have damaged red blood cell (RBC) membranes, which can lead to hemolysis associated with poor clinical outcomes. Cell-free hemoglobin is a toxin that can enhance sepsis' role in mitigating nitric oxide, inducing oxidative tissue damage, triggering coagulation, and activating innate immune pathways.[30]

In an animal model of sepsis, Arredouani et al confirmed that HP can be a biomarker of decreasing inflammation.[31] In parallel, Schaer et al found that cell-free hemoglobin can contribute to adverse sepsis outcomes in a rat model.[32] Several research studies have correlated the relationship of circulating cell-free hemoglobin with poor clinical outcomes.[33,34]

Current Study

The current study intended to identify the key genes and pathways associated with inflammation in sepsis—using microarray technology combined with bioinformatics and key inflammation-related genes (IRGs)—to perform an enrichment analysis and evaluate the value of those genes for the diagnosis and evaluation of prognosis for patients with sepsis.

METHODS

Procedures

Study. The research team performed a genetic analysis. The study took place at the Center for Emergency and Critical Medicine at Jinshan Hospital of Fudan University in Jinshan District, Shanghai, China.

The ethics committee of Jinshan Hospital of Fudan University (Approval number: JIEC 2021-S40) approved the study's protocols. The research team conducted the study in accordance with the Declaration of Helsinki, as revised in 2013.

Groups. For data analysis, the research team created two groups, the sepsis group, individuals with sepsis, and the control group, individuals without sepsis, using data for those groups from five microarray datasets obtained from the Gene Expression Omnibus (GEO) database.[35] The GSE13904 dataset divided the sepsis group into two subgroups, the sepsis and septic shock groups.

Data acquisition. The research team used GEO to obtain access to the five datasets: GSE57065, GSE28750, GSE9692, GSE13904, and GSE54514. The team annotated the first four based on the GPL570 platform and GSE54514 based on the GPL6947 platform. Table 1 provides the details of the data sets.

The team: (1) normalized all of them using the R software package Linear Models for Microarray and RNA-Seq Data (limma); (2) carried out principal component analysis (PCA) to verify data repeatability, and (3) generated PCA plots using two R software packages, FactoMineR and factoextra.

Differentially expressed genes (DEGs) analysis. To determine which genes in the datasets GSE57065, GSE28750, and GSE9692 were associated with the control and sepsis groups, the research team used the R software package limma. The team: (1) defined DEGs as those genes with an adjusted $P < .05$ and a |log2Foldchange| >1; (2) used R software package ggplot2 to create volcano plots; (3) used Venn plots to obtain the common downregulated and upregulated genes in the GSE57065, GSE28750, and GSE9692 datasets; and (4) defined the groups of frequently up- and downregulated genes in sepsis as the DEGs.

Additionally, the team retrieved 303 genes associated with IRGs from the gene sets for "Hallmark inflammatory response" and "GOBP inflammatory response" in the Molecular Signatures database (MSigDB).[36] The team used Venn plots to obtain the intersection of DEGs and IRGs, which identified nine differentially expressed IRGs (DEIRGs).

Protein-protein interaction (PPI) network and gene identification. The research team: (1) entered the DEGs into the Search Tool for the Retrieval of Interacting Genes (STRING) database[37] to create the PPI network; (2) used the Cytoscape software, version 3.8.1 (Institute of Systems Biology, Seattle, Washington, USA), to import the STRING analysis data and applied the maximal clique centrality (MCC) method of the cytoHubba plugin in Cytoscape to tag the top 20 genes as hub genes; (3) identified the overlapping hub genes and DEIRGs, finding five hub IRGs; and (4) visualized the expression of the hub IRGs for the control and sepsis groups in the GSE57065, GSE28750, and GSE9692 datasets using the R software package ggpubr.

Table 1. Details of Sepsis Data from the Gene Expression Omnibus (GEO) Database

Accession	Platform	Sample	Experiment Type	Sepsis Group n	Control Group n
GSE57065	GPL570	Peripheral blood	Array	28	25
GSE28750	GPL570	Peripheral blood	Array	10	20
GSE9692	GPL571	Peripheral blood	Array	29	14
GSE13904	GPL570	Peripheral blood	Array	97	18
GSE54514	GPL6947	Peripheral blood	Array	37	18

Functional annotation and pathway analysis. The research team: (1) conducted a gene ontology (GO) analysis to identify the biological processes (BPs), cellular components (CCs), and molecular functions (MFs) associated with the hub IRGs; (2) identified the signaling pathways related to those genes using the Kyoto Encyclopedia of Genes and Genomes (KEGG),[38] and the R software package clusterProfiler to conduct the analyses; and (3) set the threshold for statistical significance at $P < .05$ or $P < .05$; (4) used the R software package ggplot2 to display the GO and KEGG enrichment findings; (5) used the CIBERSORT computational method to estimate the Tumor-Infiltrating lmmune Cells (TIC) in the GSE57065 dataset; (6) filtered the quality data to choose only samples with $P < .05$; and (7) analyzed the correlations between the hub IRGs and the TIC abundance using Spearman correlation analysis, visualizing them using the R software package ggplot.

Comparison of expression values and receiver operating characteristic (ROC). The research team: (1) compared the expression values of the hub IRGs among the control, sepsis, and septic shock groups of the GSE13904 dataset using the Kruskal-Wallis test; (2) visualized that data using the R software package ggpubr; (3) analyzed the ROC curves using R software package pROC for hub IRGs to analyze their diagnostic accuracy in the GSE13904 dataset.

Survival analysis. The research team performed survival analysis for the hub IRGs in the GSE54514 dataset using the survminer and survival R packages.

Gene-expression evaluation in tissue. To acquire gene expressions in tissue, the research team submitted the IRGs to the Human Protein Atlas database.[39]

Outcome Measures

The research team: (1) downloaded the GSE57065, GSE28750, GSE9692, GSE13904, and GSE54514 datasets

Figure 1. Volcano Plots, Principal Component Analyses (PCAs) of Genes, and Venn Diagrams of the Hub Genes that Present Comparisons of the Sepsis and Control Groups. Figure 1A shows the PCA and Figure 1B shows the volcano plot of the GSE57065; Figure 1C shows the PCA and Figure 1D shows the volcano plot of the GSE28750; Figure 1E shows the PCA and Figure 1F shows the volcano plot of the GSE9692: Figure 1G shows the Venn diagram of the hub downregulated genes in GSE57065, GSE28750, and GSE9692; Figure 1H shows the Venn diagram of the hub upregulated genes in GSE57065, GSE28750, and GSE9692; and Figure 1I shows the Venn diagram of the hub genes in the inflammation-related genes (IRGs) and differentially expressed genes (DEGs).

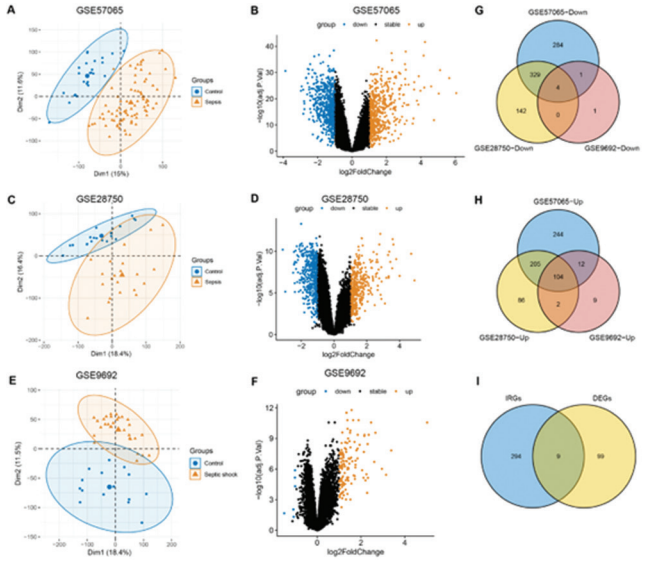
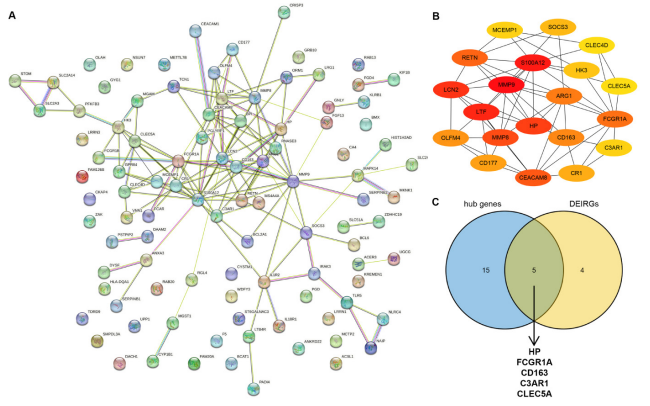


Figure 2. Analysis of Interacting Genes and Hub Inflammation-related Genes (IRGs). Figure 2A shows the STRING analysis. The research team analyzed the data and constructed the matrix using the Cytoscape software, version 3.8.1 (Figure 2B). Figure 2C shows the Venn diagrams for the Hub IRGs—HP, C3AR1, FCGR1A, CD163, and CLEC5A.



Abbreviations: C3AR1, complement C3a receptor 1; CD163, cluster of differentiation 163; CLEC5A, C-type lectin domain containing 5A; FCGR1A, Fc gamma receptor 1; HP, haptoglobin; STRING, Search Tool for the Retrieval of Interacting Genes.

from the GEO database for analysis; (2) analyzed the GSE57065, GSE28750, and GSE9692 datasets to detect the DEGs in the sepsis and control groups; (3) used Venn diagrams to obtain the intersection of DEGs and IRGs; (4) mapped the PPI network using the STRING database; (5) detected the hub genes using Cytoscape and cytoHubba; (6) performed an enrichment analysis of hub IRGs using GO and KEGG; (7) validated the expression of hub IRGs in sepsis using the GSE13904 dataset; (8) performed a survival analysis in sepsis using the GSE54514 dataset to explore the prognostic value of the hub IRGs.

RESULTS

Identification of DEGs and DEIRGs

Figures 1A, 1C, and 1E show the differences in the PCAs of the sepsis and control groups. The GSE57065 dataset provided 565 upregulated and 618 downregulated DEGs, for 1183 in total. The GSE28750 dataset provided 397 upregulated and 475 downregulated DEGs for 872 in total. The GSE9692 dataset provided 127 upregulated and 6 downregulated DEGs, for 133 in total.

Figures 1B, 1D, and 1F show the volcano plots of the upregulated and downregulated DEGs from the above datasets. Figures 1G and 1H show Venn diagrams of those three datasets, which share 4 downregulated and 104 upregulated DEGs, for 108 in total.

Figure 1I shows the Venn diagram of the intersection of DEGs and IRGs, which provided nine DEIRGs: (1) C3AR1, (2) CLEC5A, (3) interleukin 18 receptor 1 (IL18R1), (4) kinesin family member 1B (KIF1B), (5) CD163, (6) FCGR1A, (7) HP, (8) orosomucoid 1 (ORM1), and (9) vanin 1 (VNN1).

PPI Network and Gene Identification

The top 20 genes marked as hub genes (Figures 2A and 2B) included: (1) matrix metallopeptidase 9 (MMP9), (2) S100 calcium binding protein A12 (S100A12), (3) lactotransferrin (LTF), (4) lipocalin 2 (LCN2), (5) HP, (6) MMP8, (7) carcinoembryonic antigen (CEA) cell adhesion molecule 8 (CEACAM8), (8) resistin (RETN), (9) FCGR1A, (10) arginase 1 (ARG1), (11) CD163, (12) olfactomedin 4 (OLFM4), (13) CD177 molecule (CD177), (14) complement receptor type 1 (CR1), (15) suppressor of cytokine signaling 3 (SOCS3), (16) hexokinase-3 - homo sapiens (HK3), (17) mast cell-expressed membrane protein 1 (MCEMP1), (18) C3AR1, (19) CLEC4D, and (20) CLEC5A.

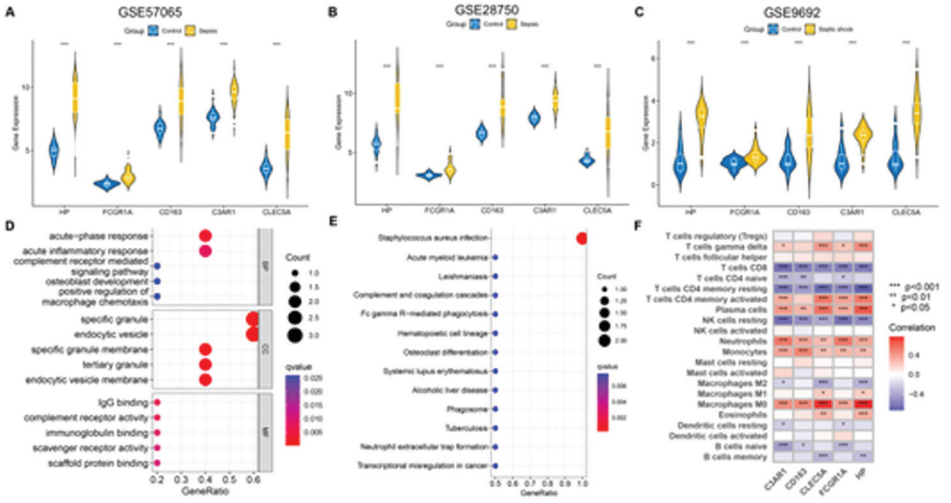
Overlapping the hub genes with the DEIRGs identified five hub IRGs: HP, FCGR1A, CD163, C3AR1, and CLEC5A (Figure 2C).

Functional Annotation and Pathway Analysis

Figures 3A, 3B, and 3C show the differences in the expression of the five hub IRGs in the GSE57065, GSE28750, and GSE9692 datasets. The sepsis group's expressions of HP, FCGR1A, CD163, C3AR1, and CLEC5A in the three datasets were significantly higher than those of the control group ($P<.001$).

Considerable enrichment occurred in the hub IRGs in acute-phase response, acute inflammatory response, specific granule, specific granule membrane, endocytic vesicle membrane, tertiary granule, immunoglobulin G (IgG) binding, complement receptor activity, Ig binding, scavenger receptor activity, and scaffold protein binding (Figure 3D). Figure 3E shows that the DEGs played a significant role in *S. aureus* infection.

Figure 3. Comparison of Gene Expression in the Three Gene Expression Profiles—the GSE57065 (Figure 3A), GSE28750 (Figure 3B), and GSE9692 (Figure 3C) datasets—of the Sepsis and the Control Groups. Figures 3D shows the clustering of the biological processes (BPs), molecular functions (MFs), and cellular components (CCs) of the potential targets, determined using the ClusterProfiler package in R software. Figures 3E shows the enriched KEGG signaling pathways selected to demonstrate the primary biological actions of major potential targets. Figure 3F shows the results of TIC abundance profile in GSE57065 using the CIBERSORT computational method. Red represents a positive correlation, and blue represents a negative correlation. The darker the color, the higher the correlation.



***$P < .001$, indicating that the sepsis group's expressions of HP, FCGR1A, CD163, C3AR1, and CLEC5A in the GSE57065, GSE28750, and GSE9692 datasets were significantly higher than those of the control group

*$P < .05$, ** $P < .01$, *** $P < .01$Pindicating that the hub IRGs were significantly positively associated at varying levels with upregulation of monocytes, neutrophils, plasma cells, uncommitted (M0) macrophages, T cells CD4 memory activated, macrophages M1, eosinophils, and T cells gamma delta and were significantly negatively associated at varying levels with downregulation of resting CD8 T cells, T cells CD4 naive, resting CD4 memory T cells, resting natural killer (NK) cells, dendritic cells resting, B cells naive, B cells memory, and macrophages M2

Abbreviations: C3AR1, complement C3a receptor 1; CD163, cluster of differentiation 163; CLEC5A, C-type lectin domain containing 5A; FCGR1A, Fc gamma receptor 1; GO, gene ontology; HP, haptoglobin; IRG, inflammation-related genes; KEGG, Kyoto Encyclopedia of Genes and Genomes; TIC, Tumor-Infiltrating Immune Cells.

Hub IRGs and TIC-abundance Correlations

Profiling the 22 immune cell types in the GSE57065 dataset (Figure 3F) showed that the hub IRGs were significantly positively associated with upregulation: (1) of monocytes—C3AR1 and CD163 ($P < .001$) and CLEC5A, FCGR1A, and HP (all $P < .01$); (2) of neutrophils— CLEC5A ($P < .01$) and, C3AR1, CD163, FCGR1A, and HP (all $P < .001$); (3) of plasma cells—CD163 ($P < .01$) and C3AR1, CLEC5A, FCGR1A, and HP (all $P < .001$); (4) of uncommitted (M0) macrophages (all $P < .001$); (5) of T cells CD4 memory activated— except for CD163, C3AR1, CLEC5A, FCGR1A, and HP (all $P < .001$); (6) of macrophages M1—CLEC5A and HP (both $P < .05$); and (7) of eosinophils—CLEC5A ($P < .01$) and HP ($P < .001$).

The hub IRGs were significantly negatively associated with downregulation: (1) of resting CD8 T cells (all $P < .001$); (2) of T cells CD4 naive—C3AR1 ($P < .001$), CD163 ($P < .01$), and FCGR1A ($P < .05$); (3) of resting CD4 memory T cells (all $P < .001$); (4) of resting natural killer (NK) cells (all $P < .001$); (5) of dendritic cells resting—C3AR1 and FCGR1A (both $P < .001$); (6) of B cells naive—C3AR1 and FCGR1A (both $P < .001$) and CD163 ($P < .05$); and (7) of B cells memory—CLEC5A and HP (both $P < .001$).

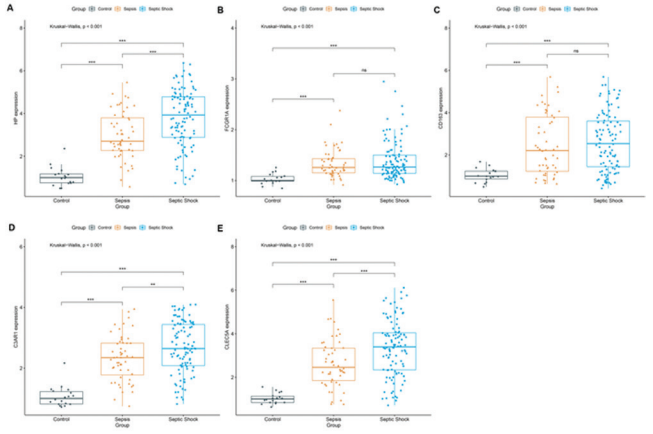
CLEC5A and HP (both $P < .001$) and C3AR1 and FCGR1A (both $P < .01$) were significantly positively correlated with T cells gamma delta, but C3AR1 ($P < .05$) and CLEC5A and HP (both $P < .001$) were significantly negatively correlated with macrophages M2.

Expression and Diagnostic Value of IRGs

The analysis validated the expression of the screened target genes in the GSE13904 dataset. A statistically significant variation existed between the sepsis and septic-shock groups ($P < .001$) for four of the IRGs—HP (Figure 4A), with $P = .00014$; C3AR1 (Figure 4D), with p=0.01; and CLEC5A (Figure 4E) with $P = .00058$. The differentiation didn't occur for FCGR1A (Figure 4B) or CD163 (Figure 4C), with $P > .05$.

Figure 5 shows that the expression of IRGs can accurately predict sepsis: (1) HP: AUC = 0.956, 95% CI: 0.924-0.988; (2) FCGR1A: AUC= 0.895, 95% CI: 0.827-0.963; (3) CD163: AUC = 0.838, 95% CI: 0.774-0.901; (4) C3AR1: AUC = 0.953, 95% CI: 0.913-0.993; and (5) CLEC5A: AUC = 0.951, 95% CI: 0.920-0.981.

Figure 4. Hub Inflammation-related Genes (IRGs) Detected in GSE13904, With Differences Between the Sepsis and Control Groups. Except for FCGR1A and CD16, significant differences existed between the sepsis and septic-shock groups. Figure 4A shows the HP expression in GSE13904; Figure 4B shows the FCGR1A expression in GSE13904; Figure 4C shows the CD163 expression in GSE13904; Figure 4D shows the C3AR1 expression in GSE13904; and Figure 4E shows the CLEC5A expression in GSE13904.



*$P < .05$, ** $P < .01$, *** $P < .01$, indicating that the septic-shock group's expressions of HP, C3AR1, and CLEC5A in the GSE13904 datasets were significantly higher than those of the sepsis group

Abbreviations: C3AR1, complement C3a receptor 1; CD163, cluster of differentiation 163; CLEC5A, C-type lectin domain containing 5A; FCGR1A, Fc gamma receptor 1; HP, haptoglobin.

Survival Analysis

According to the survival analysis, expression of HP (Figure 6A), with $P = .043$, and CLEC5A (Figures 6E), with $P < .001$, significantly improved the survival rate of participants in the sepsis group. However, participants with FCGR1A (Figure 6B), CD163 (Figure 6C), and C3AR1 (Figure 6D) expression demonstrated no significant improvement in survival time.

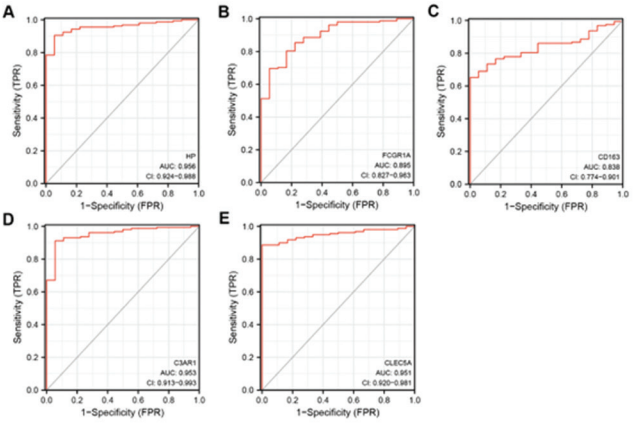
IRG Expression in Tissue

HP had a high expression of mRNA and protein in the liver, but the protein expression didn't occur in other tissues (Table 2). CD163 had a high protein expression in the lungs, but the protein expression didn't occur in other tissues. C3AR1 showed a medium protein expression in the lung and a low expression in the heart muscle and colon. No protein expression was detected in any tissue for FCGR1A.

DISCUSSION

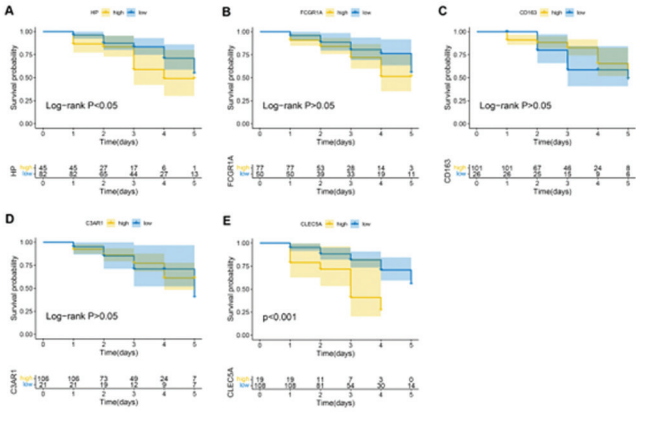
All of the hub IRGs had a high diagnostic value and may provide wide prospects in clinical application. In addition, HP, C3AR1, and CLEC5A in the GSE13904 dataset exhibited a

Figure 5. Area Under the Receiver Operating Characteristic (ROC) Curve for the Diagnosis of Sepsis. Figure 5A shows the area for HP—0.956 (95% CI: 0.924-0.988); Figure 5B the area for FCGR1A—0.895 (95% CI: 0.827-0.963); Figure 5C shows the area for CD163—0.838 (95% CI: 0.774-0.901); Figure 5D shows the area for C3AR1—0.953 (95% CI: 0.913-0.993); and Figure 5E shows the area for CLEC5A—0.951 (95% CI: 0.920-0.981).



Abbreviations: C3AR1, complement C3a receptor 1; CD163, cluster of differentiation 163; CLEC5A, C-type lectin domain containing 5A; FCGR1A, Fc gamma receptor 1; FPR, false positive rate; HP, haptoglobin; TPR, true positive rate.

Figure 6. Survival Probability Curves for Participants in the Sepsis Group with Different Protein Expressions—Log-rank Test and Pairwise Comparisons: Sepsis and Control Groups. Figure 6A shows the probability with HP expression; Figure 6B shows the probability with FCGR1A expression; Figure 6C shows the probability with CD163 expression; Figure 6D shows the probability with C3AR1 expression; and Figure 6E shows the probability with CLEC5A expression.



***$P < .05$, indicating the expression of HP and CLEC5A significantly improved the survival rate of participants in the sepsis group

Abbreviations: C3AR1, complement C3a receptor 1; CD163, cluster of differentiation 163; CLEC5A, C-type lectin domain containing 5A; FCGR1A, Fc gamma receptor 1; HP, haptoglobin.

Table 2. Inflammation-related Genes (IRG) Expression in Tissue

	Lung		Liver		Kidney		Heart Muscle		Colon	
	Protein	**RNA (nTPM)**	**Protein**	**RNA (nTPM)**	**Protein**	**RNA (nTPM)**	**Protein**	**RNA (nTPM)**	**Protein**	**RNA (nTPM)**
HP	ND	91.3	high	61113.6	ND	19.8	ND	146.7	ND	3.6
FCGR1A	ND	28.6	ND	4.2	ND	2.8	ND	2.2	ND	2.3
CD163	high	131.7	ND	89.3	ND	27.3	ND	52.7	ND	48.7
C3AR1	medium	18.6	ND	10.8	ND	3.1	low	4.4	low	7.4
CLEC5A	ND	4	ND	1.8	ND	0.5	ND	0.5	ND	0.2

Abbreviations: C3AR1, complement C3a receptor 1 (human); CD163, cluster of differentiation 163; FCGR1A, high affinity immunoglobulin gamma Fc receptor I; HP, haptoglobin; ND, not detected; nTPM, normalized transcript per million.

statistically significant variation between the sepsis and septic-shock groups, which may be related to the severity of sepsis.

The core cause of sepsis is a pathogen's infection of a host. Systemic inflammatory response or immune disorder may lead to rapid disease progression and even death. Understanding the pathogen–host relationship may benefit in the diagnosis and treatment of sepsis. As Naber previously indicated,[11] the present study found that *S. aureus* was one of the most common blood isolates during sepsis. As previously mentioned,[14] C3AR1 and FCGR1A are key genes in the sepsis infected by *S. aureus*. This highly suggests that they may contribute to the occurrence of sepsis after *S. aureus* infection. According to published studies, C3AR1 causes death of neutrophils, Neutrophils are important innate immune cells after bacterial infection. Death of neutrophils may contribute to the progression of sepsis.This suggests that he may be one of the key factors in the pathological process of sepsis. FCGR1A is a receptor for most immune cells,[16] After infection, its expression increased significantly, suggesting that this may be a good biomarker for early diagnosis.

The current study found that the sepsis group had significantly higher levels of C3AR1, FCGR1A, CD163, CLEC5A, and HP expression than the control group did. Sepsis may have a new research target with this finding. As a feature of tissues' responses to inflammation, macrophages express CD163, which indirectly participates in anti-inflammatory processes. During inflammation's progression, the increasing number of CD163+ macrophages can induce the expression of IL-6 and IL-10.[40] In a sepsis setting, the expression of inflammatory factors that macrophages induce is often out of control and is also a significant reason for sepsis progressing to severe sepsis and septic shock.The increased expression of CD163 in LPS-induced sepsis suggests that CD163 can be used as one of the markers for early diagnosis of sepsis. Macrophages are also an important part of innate immunity and play a crucial role in the progression of sepsis. This also suggests that CD163 is an important target for the study of sepsis mechanism. Besides, The C-type lectin superfamily has a pattern-recognition receptor, CLEC5A, which can trigger macrophages and cause a variety of immune-inflammatory responses. However, It remains unknown the pathway that CLEC5A trigger macrophages.

The current study examined protein and mRNA expression in the organ tissue damaged in a sepsis setting—the lung, liver, heart muscle, colon, and kidney. CD163 had high expression in the lungs. In addition, C3AR1 was expressed to varying degrees in the lungs, heart muscle, and colon.We did not find significant prognostic value in them, suggesting that their high expression may not cause impairment of organ function.Further studies could aim to confirm their role in organ tissue.

Currently, researchers still lack a comprehensive understanding of the IRG expression in tissue under a sepsis setting. HP may be biomarkers for organ damage, which warrants a follow-up study to further validate the expression in the lung, heart muscle, and colon.

Although the current study's results suggest that hub IRGs have certain value in clinical practice, the specific mechanism remains elusive in sepsis. The hub IRGs may be useful as diagnostic biomarkers in sepsis, especially in sepsis infected by *S. aureus*.

Although the current research team conducted no further experimental validation, the team did validate the expression of hub IRGs and further explored their diagnostic and prognostic value. Therefore, the results may provide research and clinical application for early diagnosis and treatment targets for sepsis. It's also important to explore the roles of sepsis-related genes, which may provide a theoretical basis for understanding sepsis pathogenesis.

However, our research still has several limitations: (1) Our research differential gene results were not verified with independent data sets; (2) Expressions of CD163, CA3AR1 and HP in tissues have not been verified in sepsis; (3) Signaling pathways were not explored in our study; (4) The diagnostic value of differential genes has not been verified.

CONCLUSIONS

HP, FCGR1A, CD163, C3AR1, and CLEC5A have value for clinical application. Clinicians can use them as diagnostic biomarkers, and they provide research direction for treatment targets for sepsis.

ACKNOWLEDGMENTS

The research team would like to acknowledge the help they received from the medical and nursing staff of the Emergency Department of Jinshan hospital.

AUTHORS' DISCLOSURE STATEMENT

The authors declare that they have no conflicts of interest related to the study. The Mass Chemical Injury First-aid, Core Discipline Improvement Project, 3-year (2020-2022) Action Plan of the Shanghai Public Health System Development (No: GWV-10.1-XK26); the Emergency and Critical Care Center, Discipline Platform Improvement Project, 3-year Talents Echelon Action Plan of Jinshan Hospital (No. XKPT-2020-3 Budget No. 1257); and the Youth Research Initial Fund of Jinshan Hospital of Fudan University (No. JYQN-LC-202109) supported the study.

DATA AVAILABILITY

Raw data and R software code are available. To obtain the raw data and R software code, send emails to gp9106@qq.com.

REFERENCES

1. Fernando SM, Rochwerg B, Seely AJE. Clinical implications of the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *CMAJ*. 2018;190(36):E1058-E1059. doi:10.1503/cmaj.170149
2. Singer M, Deutschman CS, Seymour CW, et al; The Third International Consensus Definitions for Sepsis and Septic Shock. (Sepsis-3). Jama-. *JAMA*. 2016;315(8):801-810. doi:10.1001/jama.2016.0287
3. Zhang YY, Ning BT. Signaling pathways and intervention therapies in sepsis. *Signal Transduct Target Ther*. 2021;6(1):407. doi:10.1038/s41392-021-00816-9
4. Fleischmann C, Scherag A, Adhikari NK, et al; International Forum of Acute Care Trialists. Assessment of Global Incidence and Mortality of Hospital-treated Sepsis. Current Estimates and Limitations. *Am J Respir Crit Care Med*. 2016;193(3):259-272. doi:10.1164/rccm.201504-0781OC
5. Paoli CJ, Reynolds MA, Sinha M, Gitlin M, Crouser E. Epidemiology and Costs of Sepsis in the United States-An Analysis Based on Timing of Diagnosis and Severity Level. *Crit Care Med*. 2018;46(12):1889-1897. doi:10.1097/CCM.0000000000003342
6. Evans L, Rhodes A, Alhazzani W, et al. Surviving sepsis campaign: international guidelines for management of sepsis and septic shock 2021. *Intensive Care Med*. 2021;47(11):1181-1247. doi:10.1007/s00134-021-06506-y
7. Zhang W, Wang W, Hou W, et al; The diagnostic utility of IL-10, IL-17, and PCT in patients with sepsis infection. Front Public Health. 2022;10(923457. doi:10.3389/fpubh.2022.923457
8. Jedynak M, Siemiatkowski A, Milewski R, Mroczko B, Szmitkowski M. Diagnostic effectiveness of soluble triggering receptor expressed on myeloid cells-1 in sepsis, severe sepsis and septic shock. *Arch Med Sci*. 2019;15(3):713-721. doi:10.5114/aoms.2018.73090
9. Liu AC, Patel K, Vunikili RD, et al. Sepsis in the era of data-driven medicine: personalizing risks, diagnoses, treatments and prognoses. *Brief Bioinform*. 2020;21(4):1182-1195. doi:10.1093/bib/bbz059
10. Huang M, Cai S, Su J. The Pathogenesis of Sepsis and Potential Therapeutic Targets. *Int J Mol Sci*. 2019;20(21):5376. doi:10.3390/ijms20215376
11. Naber CK; Staphylococcus aureus bacteremia: epidemiology, pathophysiology, and management strategies. Clin Infect Dis. 2009;48 Suppl 4(S231-S237. doi:10.1086/598189
12. Cheung GYC, Bae JS, Otto M. Pathogenicity and virulence of *Staphylococcus aureus. Virulence*. 2021;12(1):547-569. doi:10.1080/21505594.2021.1878688
13. Pidwill GR, Gibson JF, Cole J, Renshaw SA, Foster SJ; The Role of Macrophages in Staphylococcus aureus Infection. Front Immunol. 2020;11(620339. doi:10.3389/fimmu.2020.620339
14. Rungelrath V, DeLeo FR. *Staphylococcus aureus*, Antibiotic Resistance, and the Interaction with Human Neutrophils. *Antioxid Redox Signal*. 2021;34(6):452-470. doi:10.1089/ars.2020.8127
15. Brennan FH, Jogia T, Gillespie ER, et al. Complement receptor C3aR1 controls neutrophil mobilization following spinal cord injury through physiological antagonism of CXCR2. *JCI Insight*. 2019;4(9):e98254. doi:10.1172/jci.insight.98254
16. Bournazos S, Ravetch JV. Anti-retroviral antibody FcγR-mediated effector functions. *Immunol Rev*. 2017;275(1):285-295. doi:10.1111/imr.12482
17. Boesch AW, Brown EP, Ackerman ME. The role of Fc receptors in HIV prevention and therapy. *Immunol Rev*. 2015;268(1):296-310. doi:10.1111/imr.12339
18. Cid J, Aguinaco R, Sánchez R, García-Pardo G, Llorente A. Neutrophil CD64 expression as marker of bacterial infection: a systematic review and meta-analysis. *J Infect*. 2010;60(5):313-319. doi:10.1016/j.jinf.2010.02.013
19. Yang H, Li L, Liu X, Zhao Y; High Expression of the Component 3a Receptor 1 (C3AR1) Gene in Stomach Adenocarcinomas Infers a Poor Prognosis and High Immune-Infiltration Levels. Med Sci Monitor. 2021;27(e927977. doi:10.12659/MSM.927977
20. Wu J, Li Y, Rendahl A, Bhargava M; Novel Human FCGR1A Variants Affect CD64 Functions and Are Risk Factors for Sarcoidosis. *Front Immunol*. 2022;13(841099. doi:10.3389/fimmu.2022.841099
21. Barros MH, Hauck F, Dreyer JH, Kempkes B, Niedobitek G. Macrophage polarisation: an immunohistochemical approach for identifying M1 and M2 macrophages. *PLoS One*. 2013;8(11):e80908. doi:10.1371/journal.pone.0080908
22. Guo L, Akahori H, Harari E, et al. CD163+ macrophages promote angiogenesis and vascular permeability accompanied by inflammation in atherosclerosis. *J Clin Invest*. 2018;128(3):1106-1124. doi:10.1172/JCI93025
23. Zaki MA, Wada N, Ikeda J, et al. Prognostic implication of types of tumor-associated macrophages in Hodgkin lymphoma. *Virchows Arch*. 2011;459(4):361-366. doi:10.1007/s00428-011-1140-8
24. Skytthe MK, Graversen JH, Moestrup SK. Targeting of CD163^{+} Macrophages in Inflammatory and Malignant Diseases. *Int J Mol Sci*. 2020;21(15):5497. doi:10.3390/ijms21155497
25. Chen ST, Liu RS, Wu MF, et al. CLEC5A regulates Japanese encephalitis virus-induced neuroinflammation and lethality. *PLoS Pathog*. 2012;8(4):e1002655. doi:10.1371/journal.ppat.1002655
26. Chen DY, Yao L, Chen YM, et al. A potential role of myeloid DAP12-associating lectin (MDL)-1 in the regulation of inflammation in rheumatoid arthritis patients. *PLoS One*. 2014;9(1):e86105. doi:10.1371/journal.pone.0086105
27. Tosiek MJ, Groesser K, Pekcec A, Zwirek M, Murugesan G, Borges E; Activation of the Innate Immune Checkpoint CLEC5A on Myeloid Cells in the Absence of Danger Signals Modulates Macrophages' Function but Does Not Trigger the Adaptive T Cell Immune Response. J Immunol Res. 2022;2022(9926305. doi:10.1155/2022/9926305
28. Chen ST, Li FJ, Hsu TY, et al. CLEC5A is a critical receptor in innate immunity against Listeria infection. *Nat Commun*. 2017;8(1):299. doi:10.1038/s41467-017-00356-3
29. Sung PS, Yang SP, Peng YC, Sun CP, Tao MH, Hsieh SL. CLEC5A and TLR2 are critical in SARS-CoV-2-induced NET formation and lung inflammation. *J Biomed Sci*. 2022;29(1):52. doi:10.1186/s12929-022-00832-z
30. Remy KE, Cortés-Puch I, Solomon SB, et al. Haptoglobin improves shock, lung injury, and survival in canine pneumonia. *JCI Insight*. 2018;3(18):e123013. doi:10.1172/jci.insight.123013
31. Arredouani MS, Kasran A, Vanoirbeek JA, Berger FG, Baumann H, Ceuppens JL. Haptoglobin dampens endotoxin-induced inflammatory effects both in vitro and in vivo. *Immunology*. 2005;114(2):263-271. doi:10.1111/j.1365-2567.2004.02071.x
32. Schaer CA, Jeger V, Gentinetta T, et al. Haptoglobin treatment prevents cell-free hemoglobin exacerbated mortality in experimental rat sepsis. *Intensive Care Med Exp*. 2021;9(1):22. doi:10.1186/s40635-021-00387-7
33. Janz DR, Bastarache JA, Peterson JF, et al. Association between cell-free hemoglobin, acetaminophen, and mortality in patients with sepsis: an observational study. *Crit Care Med*. 2013;41(3):784-790. doi:10.1097/CCM.0b013e3182741a54
34. Adamzik M, Hamburger T, Petrat F, Peters J, de Groot H, Hartmann M. Free hemoglobin concentration in severe sepsis: methods of measurement and prediction of outcome. *Crit Care*. 2012;16(4):R125. doi:10.1186/cc11425
35. Gene Expression Omnibus (GEO) database. https://www.ncbi.nlm.nih.gov/geo/
36. Molecular Signatures database (MSigDB).http://www.gsea-msigdb.org/
37. Search Tool for the Retrieval of Interacting Genes (STRING) database.https://string-db.org/
38. Kyoto Encyclopedia of Genes and Genomes (KEGG). https://www.genome.jp/kegg/
39. Human Protein Atlas database. https://www.proteinatlas.org
40. Etzerodt A, Moestrup SK. CD163 and inflammation: biological, diagnostic, and therapeutic aspects. *Antioxid Redox Signal*. 2013;18(17):2352-2363. doi:10.1089/ars.2012.4834