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ORIGINAL RESEARCH

Novel Identification of CD74 as a Biomarker for Diagnosing and Prognosing Sepsis Patients

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Purpose: Sepsis, a life-threatening inflammatory condition due to an imbalanced response to infections, has been a major concern. Necroptosis, a newly discovered programmed cell death form, plays a crucial role in various inflammatory diseases. Our study aims to identify necroptosis - related genes (NRGs) and explore their potential for sepsis diagnosis.

Patients and methods: We used weighted gene co-expression network analysis to identify gene modules associated with sepsis. Cox regression and Kaplan-Meier methods were employed to assess the diagnostic and prognostic value of these genes. Single-cell and immune infiltration analyses were carried out to explore the immune environment in sepsis. Plasma CD74 protein levels were quantified in our samples, and relevant clinical data from electronic patient records were analyzed for correlation.

Results: CD74 was identified through the intersection of the hub genes of sepsis and NRGs related modules. Septic patients had lower CD74 expression compared to healthy controls. The CD74-based diagnostic model showed better performance in the training dataset (AUC, 0.79 [95% CI, 0.75–0.84]), was cross-validated in external datasets, and demonstrated better performances than other published diagnostic models. Pathway analysis and single-cell profiling supported further exploration of CD74-related inflammation and immune response in sepsis.

Conclusion: This study presents the first quantitative assessment of human plasma CD74 in sepsis patients. CD74 levels were significantly lower in the sepsis cohort. CD74 warrants further exploration as a potential prognostic and therapeutic target for sepsis.

Keywords: CD74, immunity, diagnosis, sepsis, prognosis

Introduction

Sepsis is a life-threatening condition characterized by multi-organ dysfunction resulting from a dysregulated immune response to infection.[1] Prominently characterized by organ hypoperfusion, sepsis is accompanied by systemic inflammatory response syndrome (SIRS), which can lead to extensive tissue damage.[2] Sepsis carries a high mortality rate and leads to long-term complications,[3] including the progression of chronic diseases, impaired immune function, residual organ damage, and other factors that influence patient survival.[4] Currently, biomarkers like procalcitonin (PCT) and C-reactive protein (CRP) are commonly used in sepsis diagnosis. PCT is a well-known biomarker that increases during severe infections, including sepsis.[5] CRP, an acute-phase protein, also rises in response to inflammation.[5] However, their specificity for sepsis is low, as elevated levels can also occur in non-septic inflammatory conditions. Additionally, their prognostic accuracy is limited, often failing to reliably predict sepsis outcomes. These limitations underscore the need to unveil sepsis-related diagnostic and prognostic factors.

Necroptosis, a novel programmed cell death modality, has recently entered the spotlight in the sepsis research arena. It occurs when caspases, the enzymes typically involved in apoptosis, are inhibited, triggering an alternative pathway that leads to cell lysis and the release of pro-inflammatory mediators.[6] In sepsis, this process can exacerbate the already inflamed

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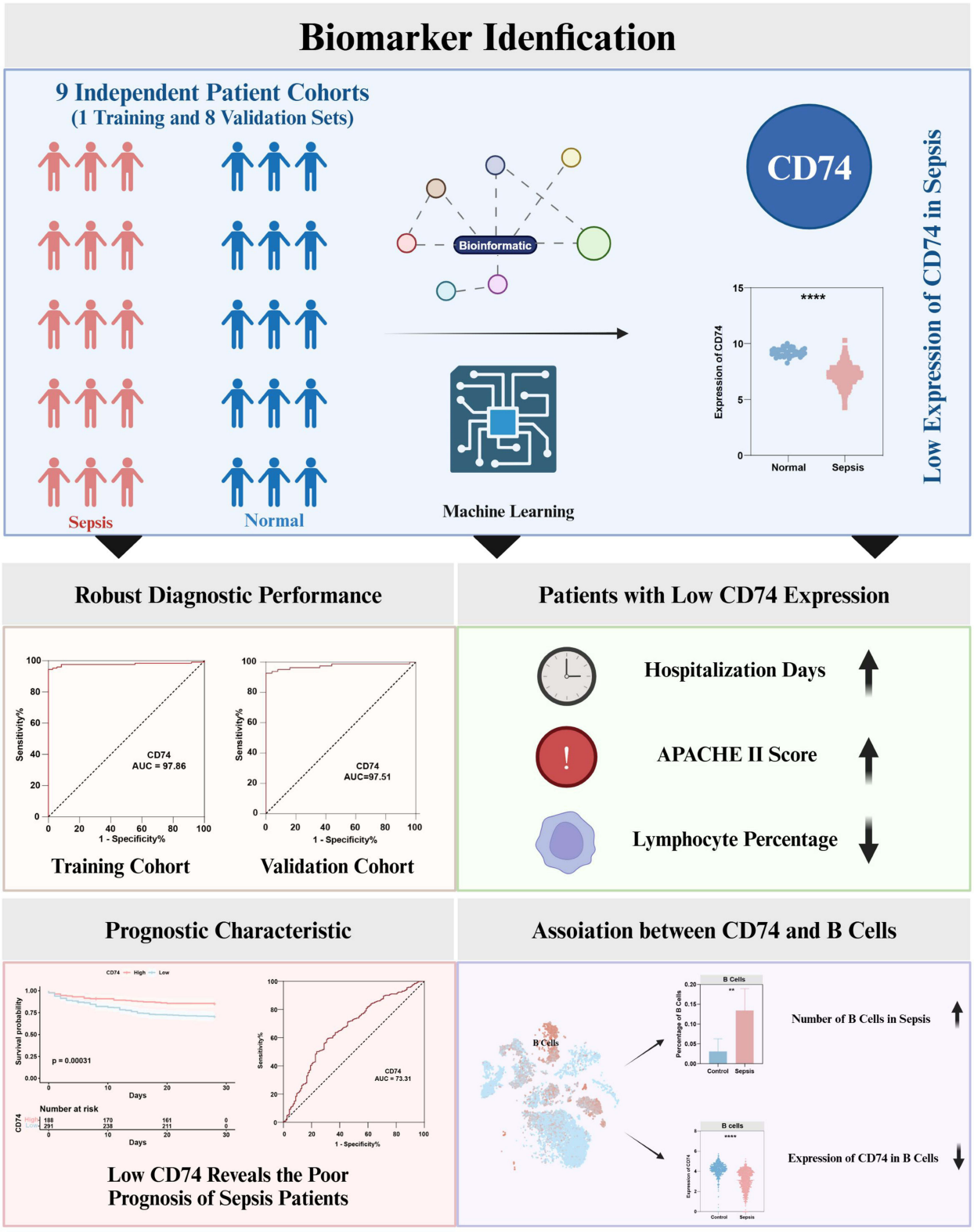
Graphical Abstract



microenvironment, potentially driving disease progression. CD74 is associated with class II major histocompatibility complex (MHC) and plays a crucial role as a chaperone in regulating antigen presentation for immune responses.[7,8] Recent research has also linked CD74 with necroptosis.[9] In the context of sepsis, CD74 may play a pivotal role in immune regulation.[10] It could be involved in antigen presentation and immune cell signaling, potentially influencing the complex inflammatory and immune responses characteristic of sepsis. Despite the known functions of CD74 in immune-related processes, research on the relationship between sepsis and necroptosis, especially with respect to CD74, remains limited. In this study, we evaluated CD74 expression with publicly available datasets at the transcriptome level and our in-house patient cohorts at the protein level. Moreover, we established a CD74-based diagnostic model and cross-validate its application in external independent datasets. Further analysis of CD74 in the diagnosis and prognosis of sepsis was supported with its potential relationship with the immune microenvironment. Our study offers new insights into sepsis from the perspective of necroptosis, providing novel findings for early diagnosis, treatment, and prognosis of sepsis patients.

Materials and Methods

Data Sources and Download

Eight public datasets of sepsis patients, including GSE65682 (760 sepsis vs 42 normal), GSE54514 (127 sepsis vs 36 normal), GSE95233 (102 sepsis vs 22 normal), GSE26440 (98 sepsis vs 32 normal), GSE57065 (82 sepsis vs 25 normal), GSE26378 (82 sepsis vs 21 normal), GSE123729 (31 sepsis vs 11 normal), and GSE28750 (21 sepsis vs 20 normal) were downloaded from the Gene Expression Omnibus (GEO) database with varying clinical information (https://www.ncbi.nlm.nih.gov/geo/).[11] Detailed information of patients included in the above cohorts can be found in the introduction pages of GEO. All datasets include the whole blood transcriptome of sepsis patients.

Necroptosis-related genes (NRGs) were identified by querying the GeneCards database (https://www.genecards.org/) using the keyword "necroptosis", NRGs with correlation scores exceeding 0.75 were selected.[12,13]

Clinical Sample and Data Collection

For the local sample collection, blood samples and clinical data from 52 sepsis patients in the intensive care unit (ICU) and 32 healthy individuals were collected at the Second Affiliated Hospital of Nanchang University. Ethical approval for this study was obtained from the Ethical Committee of the Second Affiliated Hospital of Nanchang University, and written informed consent was obtained from each patient. The inclusion criteria for sepsis patients in our in-house cohort are as follows: Patients who have clinically defined infections and meet at least two conditions: (i) body temperature > 38°C or < 36°C; (ii) Heart rate > 90 beats per minute; (iii) Respiratory rate > 20 breaths per minute; (iv) White blood cell count > 12000 per mm^3 or < 4000 per mm^3. The clinical variables analyzed in the study included: Age, Sex, Hospitalization days, Medical expenditure, Acute Physiology And Chronic Health Evaluation score (APACHE II), Heart rate, Mean arterial pressure (MAP), Temperature, White blood cell counts (WBC), Red blood cell counts (RBC), Hemoglobin (Hb), Platelet (PLT), Neutrophils (Neu), Lymphocyte (Lym), Creatine kinase (CK), Lactate dehydrogenase (LDH), Myoglobin (Mb), Glucose (Glu), Creatinine (Cr), and Estimated glomerular filtration rate (eGFR).

Weighted Gene Co-Expression Network Analysis

The WGCNA method was employed to establish a co-expression network of genes within the GSE65682 dataset to identify significant functional modules.[14] Hierarchical cluster analysis was facilitated using the "gplots" package (version 3.2.0) in R software. Gene correlations among samples were computed using the WGCNA algorithm. The soft threshold power (10) was optimized, and a standard non-proportional network was created. The WGCNA model was linked to external sample characteristics. Different colored modules were generated through hierarchical gene clustering using a dynamic tree-cutting approach. Subsequently, gene co-expression modules were constructed, and gene information was extracted from each module using WGCNA. Furthermore, the correlations between module eigenvectors and gene expression were calculated for module membership (MM), and genes with |MM| > 0.7 and within the clinically significant module were identified as hub genes for that module.

Differential Expression Analysis

To investigate transcriptional differences between the septic and control cohorts, we performed differential expression analysis using the "limma" package (version 3.56.2) in R.[15] This analysis identified differentially expressed genes (DEGs) in the GSE54514 dataset, comparing survivors and non-survivors at 1, 3, and 5 days post-infection. DEGs were defined by an absolute $\log_2$ fold change ($|\log_2 FC|$) greater than 0.5 for GSE54514, greater than 1 for GSE65682, and a P-value less than 0.05. To present the findings, we used the "ggplot2" package (version 3.5.2) in R to create a volcano plot and heat map, visualizing the results of the differential expression analysis.

Short Time-Series Expression Miner Analysis

The Short Time Series Expression Miner (STEM) was utilized to explore the expression trends of DEGs in sepsis patients (GSE54514) at day 1, 3, and 5.[16] Specifically, the average expression of each DEG served as input data. DEGs were categorized into different clusters based on their time-dependent expression trend changes, with significance determined at a P-value < 0.001.

Immune Infiltration Analysis

The CIBERSORT method, a commonly used algorithm, was applied to estimate the infiltration scores of 22 distinct immune cell types in each tissue sample, transforming the normalized gene expression data into corresponding immune cell matrices.[17,18] Additionally, we employed single-sample gene set enrichment analysis (ssGSEA) to gene set variation analysis, which uses unsupervised clustering of specific gene sets to assess the scores of 28 different immune cells in sepsis.[19] The genes associated with these 28 immune cell types were derived from previous studies and utilized to predict the extent of immune cell infiltration.

Enrichment Analysis

Gene Ontology (GO) enrichment results encompassing biological processes, cellular components, and molecular functions were obtained using the R software package "clusterProfiler" (version 4.8.3) The same R software package was used for Reactome enrichment analysis (https://www.reactome.org/).[20]

Single Cell Analysis

The distribution of CD74 in various immune cell clusters was analyzed using the dataset GSE167363, including 6 normal and 6 sepsis samples. We utilized the Seurat 4.0 package in R to conduct scRNA-seq data analysis.[21,22] Following quality control, we selected the top 2000 highly variable genes for further analysis. Dimensionality reduction was performed using principal component analysis, and cluster analysis was carried out using the top 20 principal components, leading to the identification of 9 major cell types: T cells, CD34- Pre-B Cell, Platelets, Natural killer (NK) cells, Neutrophils, Monocytes, Erythroblast, Common myeloid progenitor (CMP), and B cells.

Enzyme-Linked Immunosorbent Assay (ELISA)

The protein level of serum CD74 was determined using the Human HLA class II histocompatibility antigen gamma chain (CD74) kits (CSB-EL004956HU, Wuhan, China) following the manufacturer's instructions.

Statistical Analysis

All statistical analyses in our study were conducted using R software (version 4.3.1) and GraphPad Prism (version 8.0.2). We utilized the unpaired *t*-test, log-rank test, and one-way ANOVA to assess statistical differences. The Kaplan-Meier methods and log-rank test were utilized to compare the survival difference. Correlation analyses were performed using the Spearman method. A P-value of less than 0.05 was considered statistically significant throughout the analysis.

Results

Weighted Gene Co-Expression Network Analysis

In GSE65682 dataset, WGCNA was employed to identify gene modules significantly associated with sepsis in both the sepsis and healthy cohorts (Figure S1A), and a scale-free co-expression network was established (Figure S1B and S1C). The correlation coefficients between each module and the two mentioned groups were calculated, leading to the identification of 16 modules. Each module was assigned a distinct color label. Among these, the black module showed the highest correlation coefficient (r = −0.56, P-value = 3.5e-66) (Figure S1D). Additionally, in this black module, the correlation between septic-related gene and module membership (MM) was 0.66 (P-value = 1.8e-133) (Figure S1E). Furthermore, 459 genes with |MM| > 0.7 were designated as hub genes, visualized in Cytoscape, and selected for significant gene clusters using the MCODE plug-in (Figure S1F–S1K).

The Identification of CD74

Following the identification of the black gene module, differential expression analysis was conducted on survivor and non-survivor samples at days 1, 3, and 5 post-admission in GSE54514. This analysis yielded 1025 DEGs in non-survivor samples at day 1 (448 up-regulated and 577 down-regulated), 757 DEGs at day 3 (418 up-regulated and 339 down-regulated), and 612 DEGs at day 5 (367 up-regulated and 265 down-regulated) for the survivors and non-survivors in GSE54514. The volcano plots represented these results (Figure S2A–S2C). Heat maps were generated to display DEGs at each time point (Figure S2D–S2F). These identified DEGs were subsequently used in further analyses.

Moreover, STEM software was used to categorize the DEGs of GSE54514 into distinct modules to elucidate their expression trends in the development and progression of sepsis, with each module assigned a unique number. Fifteen modules were identified, with modules 11, 12, and 3 deemed significant (P-value < 0.001, Figure S3A).

Subsequently, an intersection analysis of the black module in WGCNA of GSE65682, module 3 in STEM of GSE54514, and the 162 necroptosis-related genes yielded one shared factor: CD74 was found to overlap among the hub genes in the black module, module 3, and the necroptosis-related genes (NRGs) (Figures S3B and 1A).

Evaluation of CD74 Expression in Sepsis Vs Healthy Group

In GSE65682 dataset, we observed that CD74 displayed significantly different expression between sepsis and healthy samples (Figure S4A). To obtain a robust predictive model for sepsis, the ROC analysis was performed with CD74- based model, which revealed an area under the curve (AUC) of 98.89 for CD74 in distinguishing between healthy and sepsis samples (Figure 1B). We assessed CD74 expression, and compared the CD74-based model performance in other seven independent public datasets, which yield similar results (Figures 1C–I and Figure S4B–S4H). This model applied to GSE65682 showed 96.6% accuracy with 96.6% sensitivity and 97.6% specificity (Table 1), showing outstanding ability to differentiate septic vs healthy patients. Similar results were obtained when evaluating the predictive model on the other seven datasets. Additionally, we compared the CD74-based model with other previously reported models in diagnosing sepsis. ROC analysis from our training cohort GSE65682 showed that the AUCs of the sepsis sNIP Score,[23] MetaScore,[24] Septicyte Score,[25] and FAIM3/PLAC8[26] were significantly lower than the CD74-based model in diagnosing patients with sepsis (Figure 1J). These findings underscore the expression pattern and diagnostic potential of CD74 in sepsis.

Validation of CD74 Expression in Sepsis Patients

Having established the differential transcriptional expression of CD74 in sepsis patients from public datasets, we validated the serum concentration of CD74 protein in our local plasma samples from 52 sepsis patients and 32 healthy individuals. The baseline information of the in-house cohort was exhibited in Table 2. CD74 concentrations were significantly lower in sepsis patients (P-value < 0.0001). The AUC of CD74-based model in our in-house cohort is 78.79, which is higher than the AUC of APACHE II Score (65.97), further emphasizing the robustness of CD74-based model in diagnosing sepsis patients (Figure 2A). Subsequently, in the univariate Cox regression analysis results, 9 variables exhibited statistical significance (Figure 2B and Table S1). Subsequently, adjusted with traditional risk factor

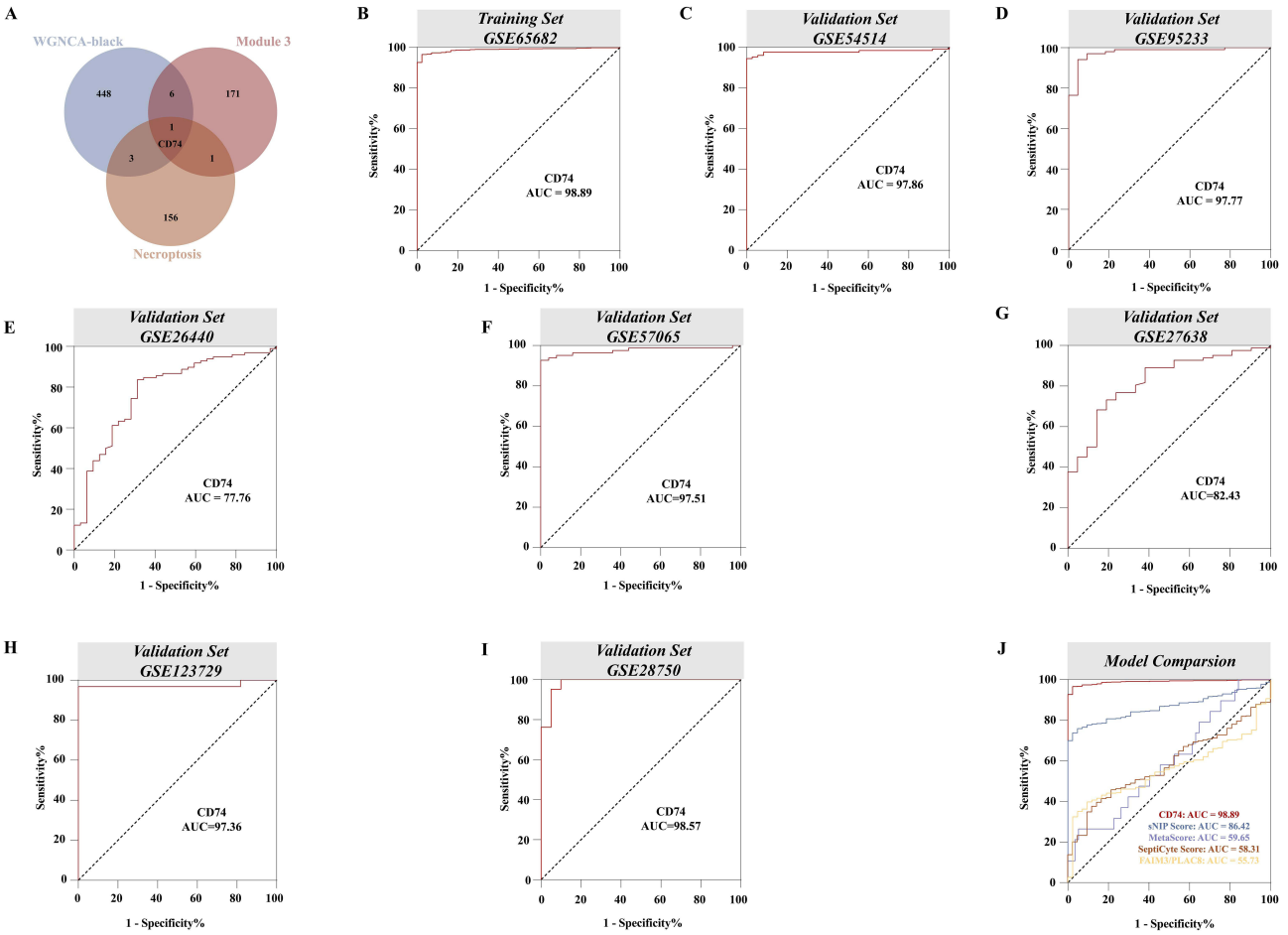


Figure 1 Determination of Necroptosis-Related Genes in Sepsis across Eight External Cohorts. (**A**) Venn diagram illustrating the overlap between hub genes in the black module, necroptosis-related genes, and Cluster No.3 identified via STEM. (**B**) The ROC curve demonstrating the diagnostic potential of CD74 expression in GSE65682 to distinguish healthy and sepsis groups. (**C**) The ROC curve demonstrating the diagnostic potential of CD74 expression in GSE54514 to distinguish healthy and sepsis groups. (**D**) The ROC curve demonstrating the diagnostic potential of CD74 expression in GSE95233 to distinguish healthy and sepsis groups. (**E**) The ROC curve demonstrating the diagnostic potential of CD74 expression in GSE26440 to distinguish healthy and sepsis groups. (**F**) The ROC curve demonstrating the diagnostic potential of CD74 expression in GSE57065 to distinguish healthy and sepsis groups. (**G**) The ROC curve demonstrating the diagnostic potential of CD74 expression in GSE27638 to distinguish healthy and sepsis groups. (**H**) The ROC curve demonstrating the diagnostic potential of CD74 expression in GSE123729 to distinguish healthy and sepsis groups. (**I**) The ROC curve demonstrating the diagnostic potential of CD74 expression in GSE28750 to distinguish healthy and sepsis groups. (**J**) The AUCs of the sNIP Score, MetaScore, Septicyte Score, and FAIM3/PLAC8 compared with the CD74 in our training cohort GSE65682.

APACHE II score and protective factor lymphocyte levels, multivariate regression suggested CD74 was an independent protective factor (Figure 2C).

In order to delve into the clinical characteristics of CD74, correlation analysis was conducted between CD74 and other clinical variables (Table S2). CD74 exhibited negative correlations with days of hospitalization, WBC, medical expenditure, APACHE II Score, Temperature, Mb, and Cr, while displaying positive correlations with eGFR, Lym, and RBC.

The Crucial Role of CD74 in the Prognosis of Sepsis

Based on our findings, we hypothesized that CD74 is significantly associated with patient prognosis and survival. We further examined potential prognostic factors, including CD74 expression levels, acquisition of infection in the intensive care unit, presence of diabetes, age, gender, and whether patients had community-acquired or hospital-acquired pneumonia, in the public patient datasets GSE65682. Only CD74 exhibited a notable impact on sepsis prognosis (P-value = 0.02, Figure S5A). Subsequently, as depicted in the prognostic heatmap (Figure S5B), survival of sepsis

Table 1 The Characteristic of CD74 in the Eight Cohorts

Cohort	Sample Counts (Sepsis/Normal)	AUC (95%CI)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
GSE65682	760/42	98.89 (0.98,0.99)	96.6	97.6	99.9	61.2	96.6
GSE54514	127/36	97.86 (0.94,0.99)	78.5	71.7	83.2	65.2	77.9
GSE95233	102/22	97.77 (0.95,1.00)	94.1	95.5	99.0	77.8	94.4
GSE26440	98/32	77.76 (0.68,0.87)	83.7	68.8	89.1	57.9	80.0
GSE57065	82/25	97.51 (0.95,1.00)	92.7	100.0	100.0	80.6	94.4
GSE26378	82/21	82.43 (0.73,0.92)	73.2	81.0	93.8	43.6	74.8
GSE123729	31/11	97.36 (0.92,1.00)	96.8	100.0	100.0	91.7	97.6
GSE28750	21/20	98.57 (0.96,1.00)	95.2	95.0	95.2	95.0	95.1

Table 2 The Characteristic of CD74 in the Eight Cohorts. The Italic Fonts Mean the Characteristic With Statistical Significance

Characteristics	Control (n = 32)	Sepsis (n = 52)	P-value
CD74, ng/mL, median (IQR)	*17.91 (15.38–21.12)*	*11.65 (2.877–16.74)*	*0.0036*
Sex, Male/Female	17/15	33/15	0.3352
Age, yr, median (IQR)	60.5 (54.25–65.25)	70 (58–76)	0.1635
Hospitalization Days, day, median (IQR)	*8 (6–9)*	*16 (8–28)*	*0.0035*
Medical Expenditure, yuan	*2031 (67.31–2272)*	*18,720 (9623–110,217)*	*0.0005*
APACHE II Score, median (IQR)	*14 (7–19)*	*3 (1–4)*	*<0.0001*
Heart Rate, per minute, median (IQR)	96 (89–105)	100 (94–113)	0.079
MAP, mmHg, median (IQR)	*54 (49–65)*	*59 (51–70)*	*0.021*
Temperature, °C, median (IQR)	*36.9 (36.5–37.1)*	*37.3 (37.0–37.8)*	*0.003*
WBC, $\times 10^9$/L	*5.61 (4.583–6.38)*	*7.05 (4.69–12.82)*	*0.0125*
RBC, $\times 10^9$/L	*4.61 (4.385–4.87)*	*3.53 (2.82–4.46)*	*<0.0001*
Hb, g/L	*138.5 (131.3–155.8)*	*100 (85–123)*	*<0.0001*
PLT, $\times 10^9$/L	*205 (173.5–232.5)*	*141 (84–212)*	*0.0163*
Neu, %	*59.95 (54.2–63.65)*	*80.7 (70.7–90.6)*	*<0.0001*
Lym, %	*32.4 (27.03–37.68)*	*12.2 (4.5–21.4)*	*<0.0001*
CK, U/L	1.345 (1.093–70.44)	99 (77.58–183.8)	0.2768
LDH, U/L	132.44 (103.17–174.9)	153.9 (143.1–193.9)	0.5673
Mb, U/L	*26.77 (18.24–42.95)*	*196.2 (152.9–689.4)*	*0.0001*
Glu, mmol/L	*5.34 (4.648–5.808)*	*8.22 (6.01–10.99)*	*0.0002*
Cr, mg/dL	*65.8 (59–79.48)*	*119 (76.83–167.5)*	*0.0234*
eGFR, mL/min/1.73m^2	*96.01 (83.74–125.3)*	*50.82 (36.2–87.77)*	*0.0003*

Abbreviations: $|\log_2 FC|$, Absolute $\log_2$ fold change; APACHE II, Acute Physiology and Chronic Health Evaluation score; AUC, Area under the curve; CK, Creatine kinase; CMP, Common myeloid progenitor; Cr, Creatinine; DEGs, Differentially expressed genes; eGFR, Estimated glomerular filtration rate; ELISA, Enzyme-Linked Immunosorbent Assay; GEO, Gene Expression Omnibus; Glu, Glucose; GO, Gene ontology; Hb, Hemoglobin; ICU, Intensive care unit; LDH, Lactate dehydrogenase; Lym, Lymphocytes; MAP, Mean arterial pressure; Mb, Myoglobin; MHC, Major histocompatibility complex; MM, Model membership; Neu, Neutrophils; NK, Natural killer cells; NRGs, Necroptosis-related genes; PLT, Platelet; RBC, Red blood cell counts; SIRS, Systemic inflammatory response syndrome; ssGSEA, Single-sample gene set enrichment analysis; STEM, Short Time Series Expression Miner; WBC, White blood cell counts.

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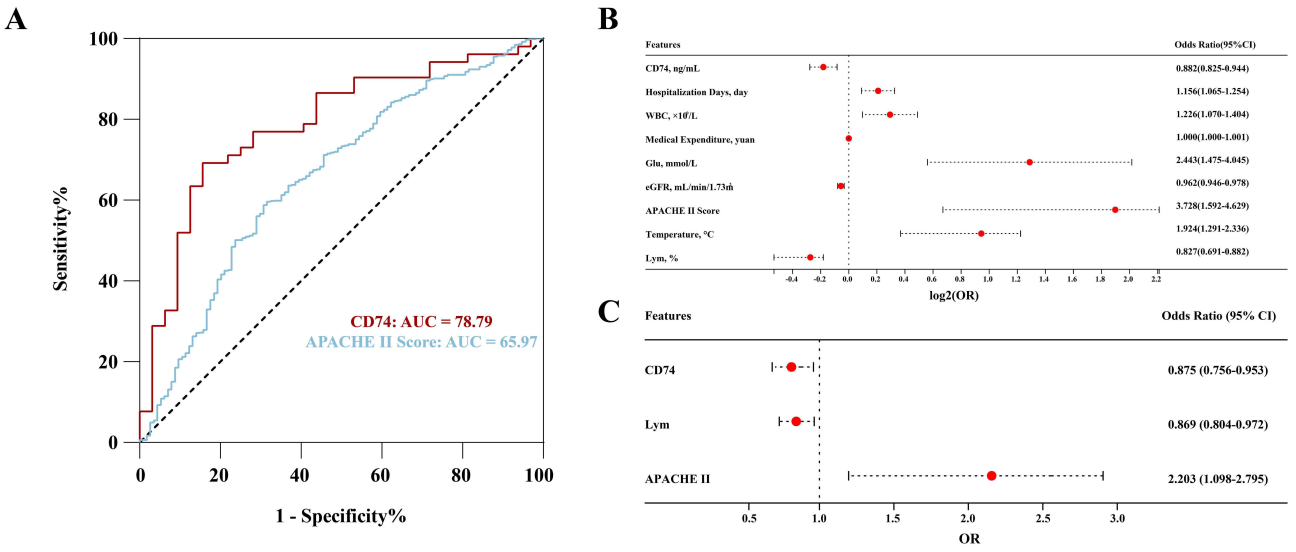

Figure 2 Crucial Diagnostic Characteristic of CD74 in our In-house Cohort. (**A**) The AUC of APACHE II Score compared with the CD74 in our in-house cohort. (**B**) The results of univariate cox regression analysis. (**C**) The results of multivariate regression analysis adjusted by age, sex, CD74 level, APACHE II score and lymphocyte values.

patients noticeably declined with increasing risk scores. As anticipated and discussed earlier, CD74 was identified as a protective factor against sepsis.

For survivorship, K-M survival curves based on CD74 expression level in sepsis patients and clinical data from GSE65682 demonstrated significant differences (P-value = 0.00031, Figure 3A). Meanwhile, CD74 also accurately distinguished survivors and non-survivors. The AUC for CD74 in distinguishing between survivors and non-survivors was 73.31 in GSE65682, 70.29 in GSE54514, 75.97 in GSE95233, 70.21 in GSE264440, and 67.79 in GSE26378 (Figures 3B–F).

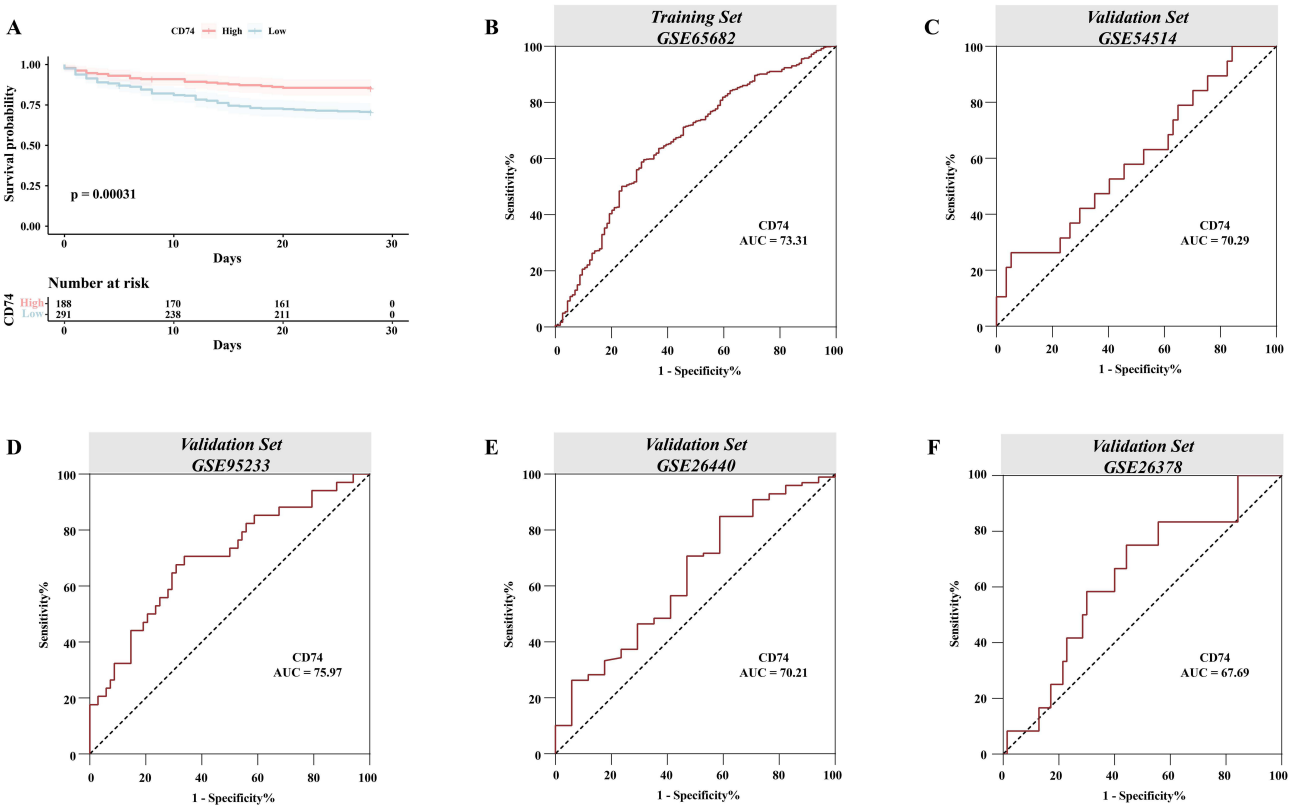

Figure 3 Prognostic Role of CD74 across the external cohorts. (**A**) Kaplan-Meier survival curves for CD74. (**B–F**) The ROC curve demonstrating the diagnostic potential of CD74 expression in all datasets to distinguish non-survivors and survivors.

Differential Expression Analysis and Enrichment Analysis Based on the Expression of CD74

To investigate the significant genes influenced by CD74 in sepsis, we categorized the samples in each dataset into two groups—high-expression and low-expression—based on the median CD74 expression levels. Differential expression analysis was then performed between these two groups. Via intersecting the DEGs in all the seven cohorts, 86 genes were identified with significant expression differences between two groups (Figure S6A–S6I).

To comprehensively investigate the specific functions and pathways associated with CD74 and these 86 genes, we conducted enrichment analysis based on the results of the differential expression analysis. We presented the top 10 pathways from the GO (BP, CC, and MF) and Reactome enrichment analyses based on the pathway-specific P-values (Figures 4A–D). Notably, all the top 10 pathways identified through these two methods were related to the MHC complex and MHC II activity.

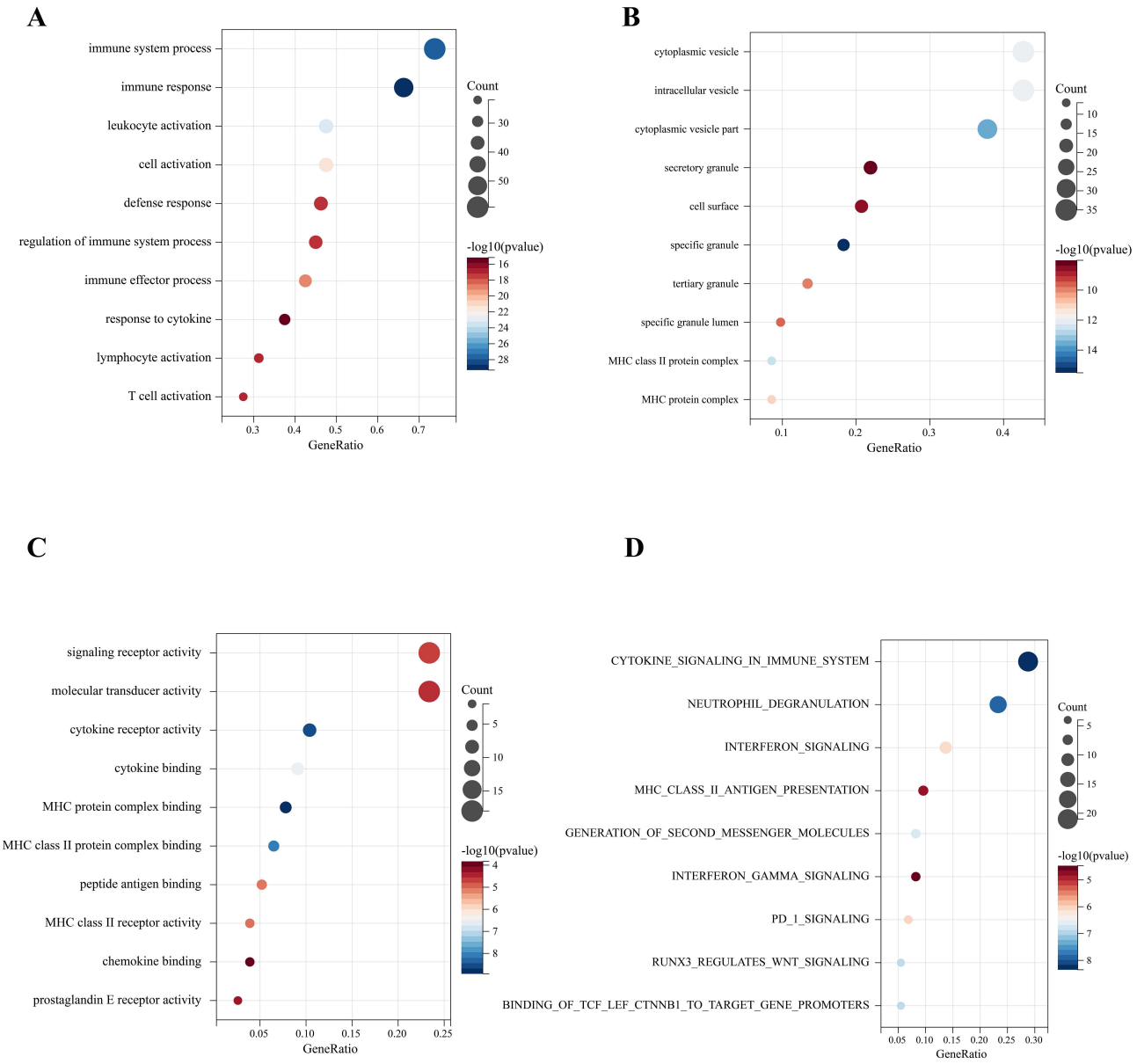

Figure 4 Enrichment Analysis for DEGs Conducted via GO and Reactome, (**A**) GO: BP, (**B**) GO: CC, (**C**) GO:MF, (**D**) Reactome.

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Investigation of the Immune Microenvironment Associated With CD74

To evaluate the immunological effects of CD74 in sepsis, we utilized immune infiltration analysis through CIBERSORT and ssGSEA. First, through the infiltration scores of various immune cells between the normal condition and sepsis we found a significant difference in the immune microenvironment in sepsis compared with the control group (Figure S7A and B). Correlation analysis between CD74 and the results of the infiltration status of various immune cells were exhibited in Figure S7C and D.

We also investigated the role of CD74 in immunity at the single-cell level. A total of 9 cell clusters were identified, including T cells, CD34- pre-B cell, Platelets, NK cells, Neutrophils, Monocytes, Erythroblasts, Common myeloid progenitor (CMP), and B cells. The distribution of cell clusters in both the healthy and sepsis groups was exhibited in Figure 5A. Meanwhile, we exhibited the expression of CD74 in each cell between the healthy and sepsis groups (Figure 5B). The expression of CD74 in each cell were exhibited, which also indicated the low CD74 expression in sepsis (Figure S7E). Moreover, based on the results of comprehensive immune infiltration and single cell analysis, we found that the increasing number of B cells is associated with a low expression of CD74 (Figures 5C and D). This may be partially explained by the correlation study that showed that CD74 is positively correlated with the number of lymphocytes (Lym, r=0.367, p-value=0.001, Table S2). This emphasized the role of CD74 in regulating the immune microenvironment.

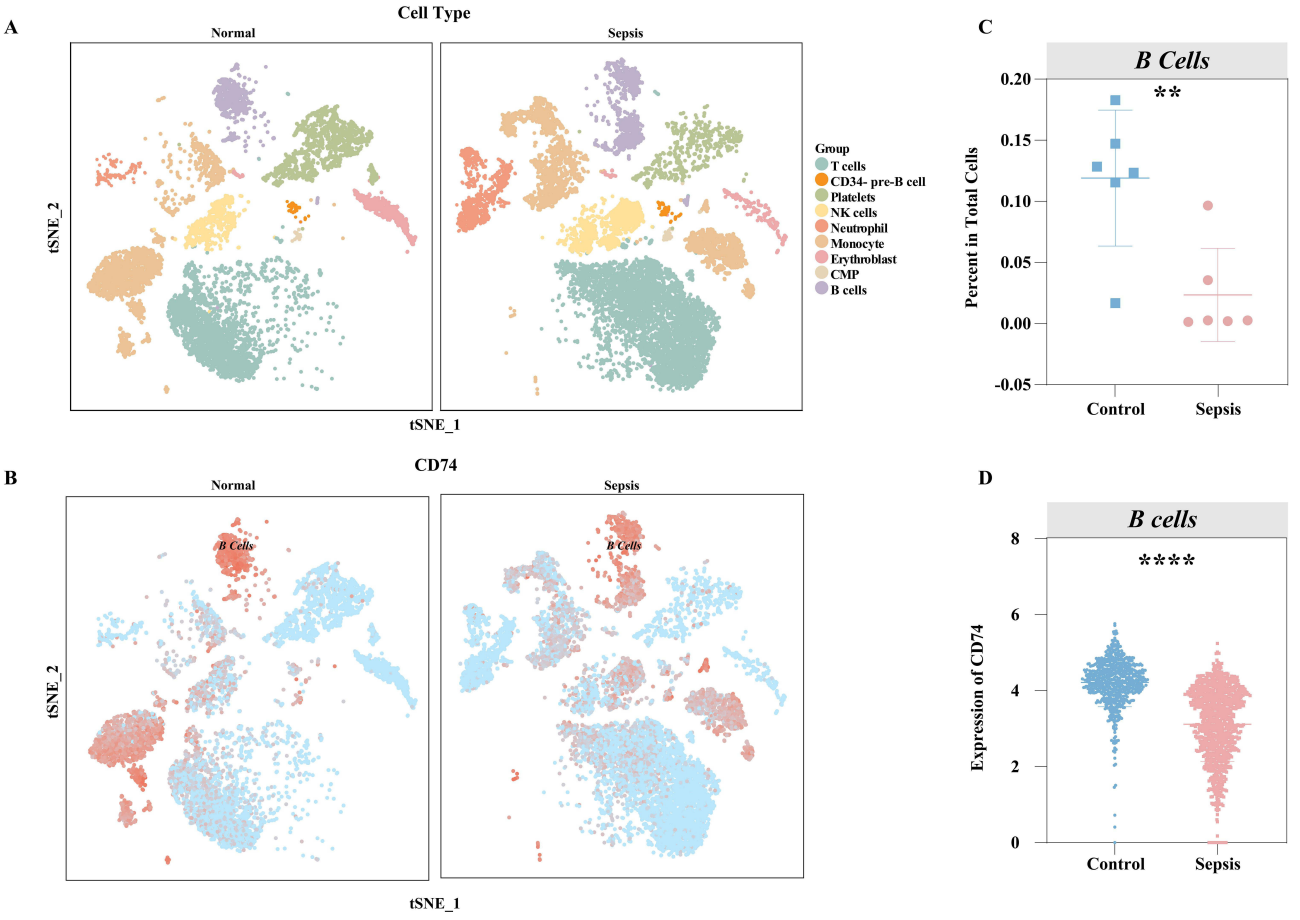

Figure 5 Landscape of CD74 in the Regulation of Immune Microenvironment of Sepsis. (**A**) The tSNE plot indicated the distribution of 9 cell clusters in the healthy and sepsis groups. (**B**) The distribution of the expression of CD74 in each cell between the healthy and sepsis groups. (**C**) The percentage of B cells between the healthy and sepsis groups. (**D**) The expression of CD74 in each cell cluster between the healthy and sepsis groups. **, P-value < 0.01; ****, P-value < 0.0001.

Discussion

Sepsis continues to exhibit a high prevalence and mortality rate, imposing a substantial burden on the public health sector.[27] Recent years have seen a renewed interest in exploring the relationship between necroptosis and sepsis.[28,29] To explore the link between necroptosis and sepsis, we conducted a series of bioinformatic and machine learning analysis in public dataset with local validation, which was exhibited in the Graphical Abstract. We identified CD74 as the most significant NRG in the diagnosis and prognosis of sepsis. Previous research has confirmed that reduced level of CD74 mRNA played a crucial role in predicting mortality of patients with septic shock. Specifically, lower expression of CD74 mRNA in septic shock patients is associated with higher mortality.[30] Meanwhile, several bioinformatics analyses regarded CD74 mRNA as a potential diagnostic factor in sepsis.[31] Intriguingly, this is the first report that validates the CD74 protein as a diagnostic marker of sepsis and explored its crucial correlational relationship to other clinical indicators. The disparities in CD74 expression and diagnostic capabilities were also confirmed through ELISA validation.

Our findings are consistent with previous research. For example, Cazalis et al found that reduced CD74 expression predicts mortality in septic shock patients.[30] In our investigation, we not only evaluated CD74 expression in publicly available GEO datasets but also validated findings in our patient cohort. Additionally, this study marks the first instance of integrating CD74 with a range of diverse common clinical variables, aiming to enhance accuracy in the diagnosis of sepsis. In addition to traditional risk factors such as lymphopenia,[32] multivariate analysis unveiled CD74 as a novel independent protective factor for sepsis. Moreover, the prognostic role of CD74 in sepsis was also indicated. The APACHE II score is a commonly used tool for assessing the degree of organ failure in critically ill patients and is used to predict patient prognosis and disease severity.[33] Sepsis patients with higher APACHE II score often face a poor prognosis. The negative correlation between CD74 and APACHE II score in our research demonstrated the predictive possibility of CD74 in the prognosis of sepsis.[34] Notably, CD74 expression exhibited a negative correlation with WBC count, indicating that individuals with low CD74 expression may experience more pronounced inflammatory reactions. Abundant studies have indicated that the lack of CD74 can promote inflammation, such as renal interstitial fibrosis and inflammation after ureteral obstruction.[35,36] Meanwhile, our research suggests that low CD74 is associated with lower eGFR in patients with sepsis. SIRS itself can damage kidney function and lead to kidney failure.[37] Activation of immune infiltration in patients with a low expression of CD74 may further exacerbate inflammation. Interestingly, sepsis patients exhibit higher Glu levels compared to normal patients, potentially indicating that severe infection may disrupt Glu control. Poorly controlled Glu can further exacerbate damage to the immune and circulatory systems, creating a vicious cycle. Higher Glu levels have been confirmed to increase mortality in sepsis patients.[38,39] Moreover, we found that expression of CD74 was negatively correlated with medical expenses and days of hospitalization, which may play a critical role in developing reasonable medical strategies and reducing patient burden.

Sepsis is an infectious disease hallmarked by inflammation and immunosuppression.[40] Central to its immune response regulation is CD74, the high-affinity receptor for macrophage, which is crucial for the immune system's balance and assist MHC class II molecules in presenting exogenous antigen peptides, and playing a crucial role in launching the humoral immune response.[41] Specially, our research revealed a positive association between CD74 levels and lymphocytes. We observed that lower levels of CD74 expression corresponded with decreased immune cell infiltration, particularly of B cells, as shown in single-cell analyses. MIF functions to block monocyte migration towards chemokines and to halt B cell migration in vitro. These functions are modulated by CXCR2 and CXCR4 under the strength of CD74.[42,43] Accordingly, reduced CD74 levels might impede MIF's immunosuppressive mechanisms, potentially worsening the prognosis for patients with sepsis. Concerning B cells in septic patients, diminished CD74 expression could relieve MIF's active effect on B cell activity, contributing to a rise in inhibitory B cells in patients with low CD74 expression. Our findings suggest that B cells may be critical in determining sepsis outcomes; a drop in CD74 expression could impair the body's capacity to eliminate sources of infection, thereby increasing mortality risk in sepsis patients. Another perspective of sepsis is cell death. While various studies have delved into the different types of cell death induced by sepsis, the specific link between CD74/MIF and necroptosis remains under-researched.[44,45] Only a single study by Soppert et al highlighted that soluble CD74 may promote necroptosis in cardiac myofibroblasts through the MIF/CXCR4/AKT pathway.[46] Despite these insights, current knowledge of the connection between CD74/MIF and

necroptosis is scarce. The interplay between CD74, MIF, and necroptosis could be complex. CD74 may regulate MIF-mediated signaling pathways that are involved in the initiation or regulation of necroptosis. For example, MIF, under the influence of CD74, may interact with other molecules in the necroptotic pathway, such as RIPK1, RIPK3, and MLKL, which are key players in the necroptotic machinery. Further studies are required to elucidate these potential interactions. Therefore, it's imperative that future studies investigate the role of CD74 in relation to necroptosis within the context of sepsis and other inflammatory conditions.

The strengths of our study include the assessment of CD74 protein expression in sepsis diagnosis, a novel composite of different clinical variables in sepsis survivors, the use of different independent cohorts and the biological plausibility based on CD74 levels. The diagnostic value of CD74 is compelling because of the increasing needs of the sepsis population in recent years, and emerging evidence suggesting that early diagnosis with timely management will significantly change the overall clinical outcomes. Our study reinforces the relevance of immunoregulatory molecules, especially CD74, in the context of sepsis.

This study has certain limitations. Firstly, the number of patient plasma samples available for clinical validation is limited. Future studies with larger sample sizes are warranted to yield more robust findings. Secondly, due to budget constraints, we did not conduct a time-series analysis. Prolonged and dynamic observations would be beneficial in understanding the significance of CD74 expression in evolving immunity during sepsis. And longitudinal data would provide insights into how CD74 levels change over the course of sepsis and recovery, which could further validate its prognostic value. Thirdly, there may exist potential factors that have not been considered and included in our study. Fourthly, the potential biological mechanisms how CD74 regulates necroptosis by MIF in sepsis are still unclear. Fifthly, the SOFA score and APACHE II Score cannot be calculated in all GEO sepsis cohorts. Therefore, we are unable to compare our model with these two clinical scoring models in the public cohort. Larger prospective studies are needed to compare CD74-based models with other clinical scoring systems.

Conclusions

CD74 expression was associated with sepsis diagnosis. The potential immunoregulatory effects of CD74 significantly influence the clinical profiles and outcomes of septic patients. Our study suggests a novel diagnostic and prognostic biomarker CD74 for sepsis; quantification of CD74 could identify possible sepsis patients and aid prompt management, improving clinical outcomes.

Data Availability

The original data used in this project can be downloaded in the public database GEO (https://www.ncbi.nlm.nih.gov/geo/). The data analyzed during the human experiments are available from the corresponding author on reasonable request.

Ethics approval and informed consent

The protocol for collecting human tissue samples was approved by Ethics Committee of the Second Affiliated Hospital of Nanchang University (No. BRAF/SG-03), and all procedures were performed in accordance with the 1964 helsinki Declaration and its later amendments or comparable ethical standards. Written informed consent was provided by all participants before their inclusion in the study.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors have no relevant financial or non-financial interests to disclose for this work.

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