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ORIGINAL RESEARCH

Identification of CD19$^+$B Cell as a Diagnostic Biomarker in Sepsis-Induced ARDS

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Background: Sepsis has a high morbidity and mortality rate in critically ill patients, and acute respiratory distress syndrome (ARDS) is one of its most common outcomes. However, there is still no effective biomarker to predict the risk and outcome of ARDS induced by sepsis.

Methods: In this research, the GSE32707 dataset was acquired from the Gene Expression Omnibus (GEO) database and used to identify differentially expressed genes (DEGs). The extracellular protein-related differentially expressed genes (EP-DEGs) were filtered using the Human Protein Atlas (HPA) and UniProt databases. Functional and pathway analyses of the EP-DEGs were conducted through Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses. Additionally, hub genes were identified using STRING, Cytoscape, MCODE, and Cytohubba. The expressions of the hub genes were analyzed in both the training set (GSE32707) and the validation set (GSE66890). The diagnostic potential of lymphocyte subsets was evaluated through ROC curve assessment in the clinical cohort.

Results: We identified 86 EP-DEGs from DEGs. These EP-DEGs were found to be significantly enriched in leukocyte mediated immunity. We also identified 5 key extracellular protein genes GNLY, GZMK, CST7, PTPRC and CD19. CD19 expressions were increased in both training and validation sets. ROC curves showed that CD19 expression had a higher accuracy in the diagnosis of sepsis-induced ARDS. Lymphocyte subsets analysis of clinical samples revealed that CD19$^+$B cells were elevated in sepsis-induced ARDS, with CD19$^+$B cell counts demonstrating a higher diagnostic accuracy (AUC = 0.829) for septic-ARDS compared to other lymphocyte subsets.

Conclusion: In this study, we employed bioinformatics approaches to identify potential biomarkers for sepsis-induced ARDS and further validated these findings using clinical samples. Our results suggest that peripheral CD19$^+$ B cells could act as a promising biomarker in sepsis-induced ARDS.

Keywords: sepsis-induced ARDS, B cell, bioinformatics analysis, predictive biomarker

Introduction

ARDS is a rapid-onset, widespread inflammatory condition affecting the lungs, triggered by various risk factors including pneumonia, infections outside the lungs, trauma, blood transfusions, burns, aspiration, or shock.[1] It was reported that the mortality rate of ARDS in critically ill patients was up to about 40% in adults and 33% in pediatric patients.[2–4]The COVID-19 pandemic has heightened the awareness of ARDS due to a sharp increased incidence of ARDS.

ARDS can be triggered by a wide range of factors, with sepsis being the most common cause.[5] Sepsis is a dysregulated immune reaction to infection, resulting in potentially fatal organ dysfunction.[6] Sepsis causes a significant inflammatory reaction that can heighten alveolar-capillary permeability, resulting in the accumulation of protein-rich edema fluid within the alveolar lumen and widespread interstitial lung edema eventually.[7–9] ARDS triggered by sepsis leads to more severe lung damage and higher mortality rates compared to ARDS not associated with sepsis.[10] Therefore, predicting the occurrence and outcomes of ARDS in septic patients is essential.

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Recently, advanced generation sequencing technology and integrated analytical approaches have been conducted to identify potential key molecules in sepsis-induced ARDS. For instance, studies have indicated that the serum levels of Myeloid-related proteins 8 and 14 (Mrp 8/14) at the time of admission could serve as a potential biomarker for predicting the development of sepsis-induced ARDS.[11] Studies have revealed that the expression levels of ATM, CCNB1, CCNA1, and E2F2 could potentially function as diagnostic biomarkers and therapeutic targets for sepsis-induced ARDS.[12] Furthermore, ferroptosis-related genes such as IL1B, MAPK3, and TXN may also act as potential diagnostic biomarkers for sepsis-induced ARDS.[13] However, no biomarker has been recommended for clinical use due to insufficient understanding of the risks and outcomes associated with ARDS in sepsis patients. As a result, there is a need to develop more predictive or prognostic stratification methods to identify potential biomarkers and novel therapeutic targets for sepsis-induced ARDS.

In this study, we obtained microarray gene expression profiles of sepsis patients and sepsis-induced ARDS patients from the GEO database (GSE32707) and utilized R software to identify differentially expressed genes (DEGs). To identify detectable biomarkers in the peripheral blood of sepsis-induced ARDS patients, we filtered extracellular protein-related differentially expressed genes (EP-DEGs) from the DEGs. We then conducted biological function and pathway enrichment analyses of these EP-DEGs using the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases. Additionally, a protein-protein interaction (PPI) network of EP-DEGs was constructed to identify functional modules, hub genes, and extracellular molecules interacting with these hub genes. Finally, peripheral blood samples from sepsis and sepsis-induced ARDS patients were collected to validate the expression of key EP-DEGs and assess their diagnostic potential for sepsis-induced ARDS. This research aims to uncover potential biomarkers for sepsis-induced ARDS and explore new therapeutic strategies for its treatment.

Materials and Methods

Dataset

The mRNA profiles of GSE32707 were acquired from the Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo). These profiles included whole blood samples collected from patients diagnosed with sepsis alone as well as those with sepsis-induced ARDS. Total RNA was extracted from whole blood samples, and the mRNA profiles were analyzed using the Illumina HumanHT-12 V4.0 expression beadchip.

Screening of DEGs and EP-DEGs

The Limma package in R was employed to identify differentially expressed genes (DEGs) within the GSE32707 dataset. $p < 0.05$ and $|\log 2 \text{ (fold change [FC])}| \geq 0.5$ were set as the threshold values of DEG identification. The UniProt database and the Human Protein Atlas (HPA) protein annotation database were utilized to extract EP-DEGs, distinguishing the sepsis-induced ARDS group from the sepsis-alone group.

Functional Enrichment Analysis and Pathway Analysis

GO and KEGG pathway enrichment analysis were accomplished in DEGs by using clusterProfiler package in R.[14] The gene set enrichment analysis (GSEA) of the central gene was conducted by the R package "clusterProfiler", with $P < 0.05$ as the significant threshold.

The PPI Network and Hub Gene Analysis

A protein-protein interaction (PPI) network of EP-DEGs was built using the STRING database (https://string-db.org/). The CytoHubba plug-in in Cytoscape was utilized to identify the top 10 node genes using five different algorithms, and a Venn diagram was employed to identify the hub genes. The mRNA expression levels of these hub genes were analyzed in the training set GSE32707 and the validation set GSE66890. Additionally, the diagnostic potential of the hub genes was evaluated using receiver operating characteristic (ROC) curves, generated with the pROC package in R.

Table 1 Clinical Characteristics of Patient Cohorts

	Sepsis (n=27)	Sepsis-ARDS (n=32)	p	Adjust-P
Age, mean±SD	31.33±22.23	46.72±13.98	0.0125*	
Gender, n (%)				
Female	12(44.44%)	12(37.5%)	0.589	0.750
Male	15(55.56%)	20(62.5%)		
WBC Count (*10^9/L)	8.15±6.42	9.40±8.91	0.676	0.622
Neutrophil Count (*10^9/L)	6.76±6.04	8.50±8.74	0.473	0.465
Lymphocytes Count (*10^9/L)	0.76±0.67	0.41±0.35	0.041*	0.022*
$CD3^+CD4^+$ Count (/uL)	328.85±376.03	177.91±191.28	0.020*	0.290
$CD3^+CD8^+$ Count (/uL)	320.07±335.43	137.59±184.32	0.019*	0.200
$CD3^-CD19^+$ Count (/uL)	22.26±19.22	54.91±28.15	<0.001***	<0.001***
$CD3^-CD\ (16^+56)^+$ Count (/uL)	98.52±134.99	43.41±37.40	0.140	0.124
Interleukin 6, IL-6 (pg/mL)	944.73±2972.4	426.92±916.2	0.039*	0.500
C Reactive protein, CRP (mg/L)	108.89±77.41	119.48±95.54	0.844	0.400
Procalcitonin, PCT (ng/mL)	8.18±24.17	10.27±23.72	0.228	0.713
Site of infection, n (%)				
Lung	19(70.37%)	23(71.88%)	0.899	0.739
Others	8(29.63%)	9(28.13%)		
Outcome, n (%)				
Alive	17(62.96%)	12(37.50%)	0.051	0.125
Death	10(37.04%)	20(62.50%)		

Note: *p < 0.05, ***p < 0.001, adjusted for age.

Immune Infiltration Analysis

The CIBERSORT tool, a deconvolution algorithm specifically developed to quantify the proportions of 22 different subtypes of infiltrating immune cells, was employed to conduct immune infiltration analysis.[15] We summarized the 22 immune cell subtypes into 10 categories and examined the differences in immune cell marker expression between the sepsis group and the sepsis-induced ARDS group.

Clinical Data Collection and Lymphocyte Subsets Analysis

We collected 27 patients with sepsis and 32 patients with sepsis-induced ARDS in the Qilu Hospital of Shandong University. This study was approved by the Ethics Committee of Qilu Hospital of Shandong University (KYLL-202202-027-1). The patients diagnosed with sepsis and sepsis-induced ARDS were identified based on the diagnostic criteria outlined in the 2016 international Sepsis 3.0 consensus, developed by sepsis experts, and the Berlin definition and diagnostic criteria for ARDS.[6,16] Clinical data of patients were recorded, including age, gender, peripheral blood cell counts, infections, and outcomes (Table 1). Peripheral blood samples were collected from each patient in 5 mL EDTA tubes on the day of enrollment. Blood samples collected more than 24 hours after intubation were excluded. Patients were also excluded if they had received an organ transplant, had active malignancy, or were undergoing systemic immunosuppression or glucocorticoid therapy. Each sample was analyzed using a multicolor flow cytometer (BD FACSCanto II) following the manufacturer's instructions.

Statistical Analysis

The limma package, ClusterProfiler package, and Cytoscape v3.9.1 software were used to analyze publicly available gene expression data. For statistical analysis of clinical data, SPSS Statistics v28.0.1.1 and GraphPad Prism 9.3.1 were employed. The diagnostic accuracy of indicators was evaluated using receiver operating characteristic (ROC) curve analysis. Continuous data were expressed as mean ± standard error of the mean (SEM) and analyzed using the Mann–Whitney *U*-test. Categorical data were presented as numbers (N) and percentages (%) and analyzed using the Chi-square test. A p-value < 0.05 was considered statistically significant.

Results

Identification of DEGs between Sepsis and Sepsis-Induced ARDS

The GSE32707 dataset was analyzed to identify DEGs between the sepsis group (30 samples) and the sepsis-induced ARDS group (18 samples). The median, upper and lower quartiles, maximum, and minimum values of gene expression across the 48 samples were mostly consistent (Figure 1A). A total of 364 DEGs were identified in sepsis compared to sepsis-induced ARDS samples, including 116 up-regulated genes and 248 down-regulated genes (Figure 1B). The heatmap of DEGs demonstrated significant differences in gene expression between the sepsis group and the sepsis-induced ARDS group (Figure 1C).

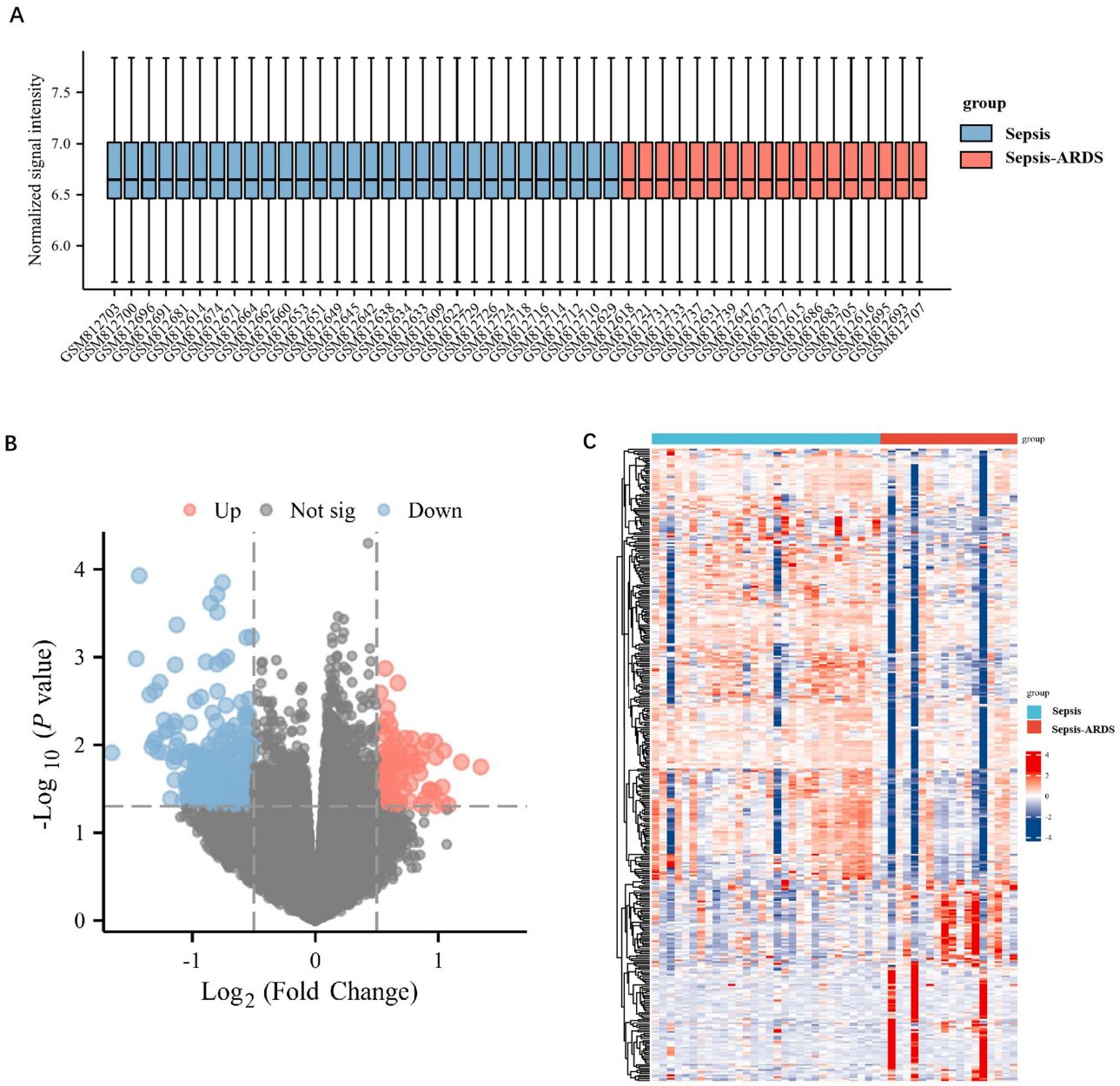

Figure 1 Identification of DEGs. (**A**) Boxplot of gene probe expression levels among samples. There was no significant difference in the median and the upper and lower quartile. (**B**) Volcano map of all DEGs in sepsis group and sepsis-induced ARDS group. (**C**) Heatmap of all DEGs in sepsis group and sepsis-induced ARDS group.

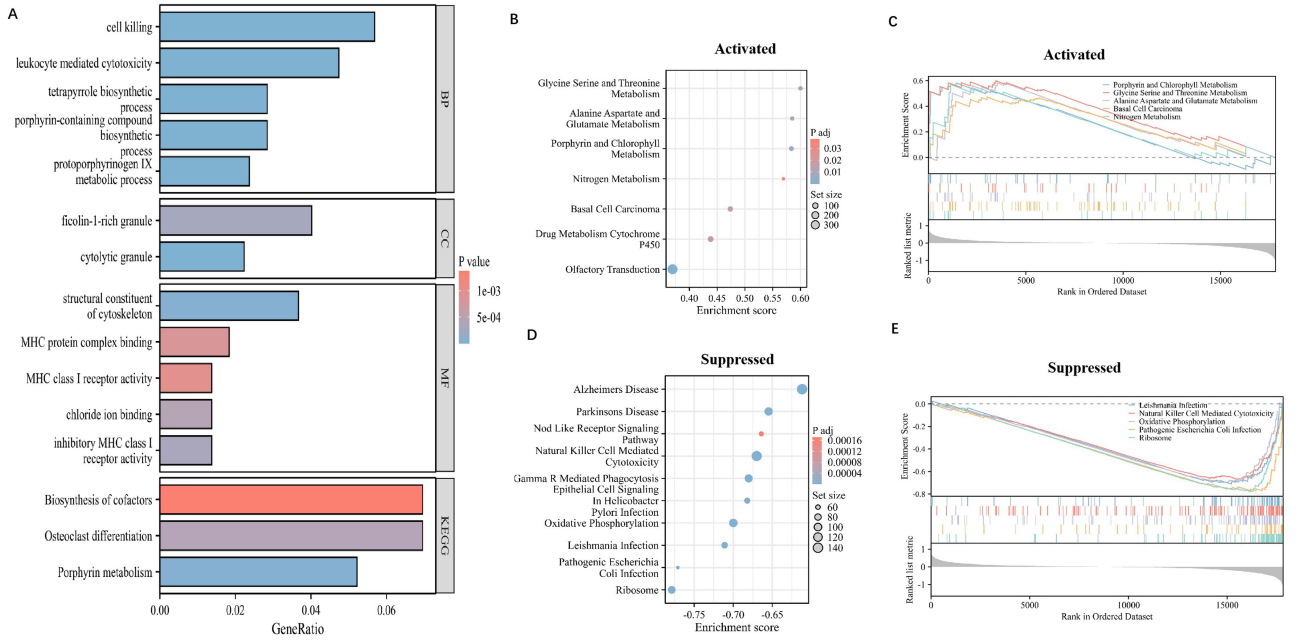

Figure 2 Functional and pathway enrichment analysis of DEGs. (**A**) GO and KEGG enrichment analyses of DEGs. (**B** and **C**) The activated pathways associated with DEGs. (**D** and **E**) The suppressed pathways associated with DEGs.

Functional and Pathway Enrichment Analysis of DEGs

Gene Ontology (GO) term was used to analyze the functional enrichment of DEGs involved in biological process (BP) closely related to the cell killing and leukocyte mediated cytotoxicity. The cellular components (CC) involved are mainly related to ficolin-1-rich granule and cytolytic granule. The molecular functions (MF) involved are mainly related to structural constituent of cytoskeleton. Combined with the results of KEGG pathway enrichment showed that DEGs were closely related to biosynthesis of cofactors, osteoclast differentiation, and porphyrin metabolism pathways (Figure 2A). We also used GSEA to investigate the up-regulation and down-regulation pathways related to DEGs. We found that the top five up-regulated pathways of DEGs enrichment were glycine serine and threonine metabolism, alanine aspartate and glutamate metabolism, porphyrin and chlorophyll metabolism, nitrogen metabolism, and basal cell carcinoma (Figure 2B and C). The top five down-regulated pathways were leishmania infection, natural killer cell mediated cytotoxicity, oxidative phosphorylation, pathogenic Escherichia coli infection, and ribosome (Figure 2D and E).

Screening of EP-DEGs

To identify genes encoding extracellular proteins that are differentially expressed between the sepsis group and the sepsis-induced ARDS group, we extracted EP-DEGs from the DEGs and conducted further analysis. As a result, 25 EP-DEGs were identified through intersection with the UniProt database, and 80 EP-DEGs were identified through intersection with the HPA database. By combining the results from both methods, 19 genes were overlapped (Figure 3A). A total of 86 EP-DEGs were identified. The top 10 up-regulated genes with the smallest p-value were CHST3, CD19, MFGE8, RG2, CCS, SLC7A5, PHGDH, CS, ZNF653, and BCR. The top 10 down-regulated genes were CCL4L2, TNPO1, PTPCR, FGFBP2, IFNAR2, CTSC, SLGLEC10, CLIC3, SH3BP1, and RHOC (Figure 3B). The clustered heatmap displayed the top 20 most significantly up-regulated and down-regulated EP-DEGs, selected based on the smallest p-values (Figure 3C).

Enrichment Analysis of EP-DEGs

EP-DEGs were mainly enriched in leukocyte mediated immunity and leukocyte activation involved in immune response of BPs, cytoplasmic vesicle lumen and secretory granule lumen of CCs, and MHC protein complex binding and MHC class I receptor activity of MFs (Figure 4A). The Cluster Profiler package was utilized to visualize the EP-DEGs

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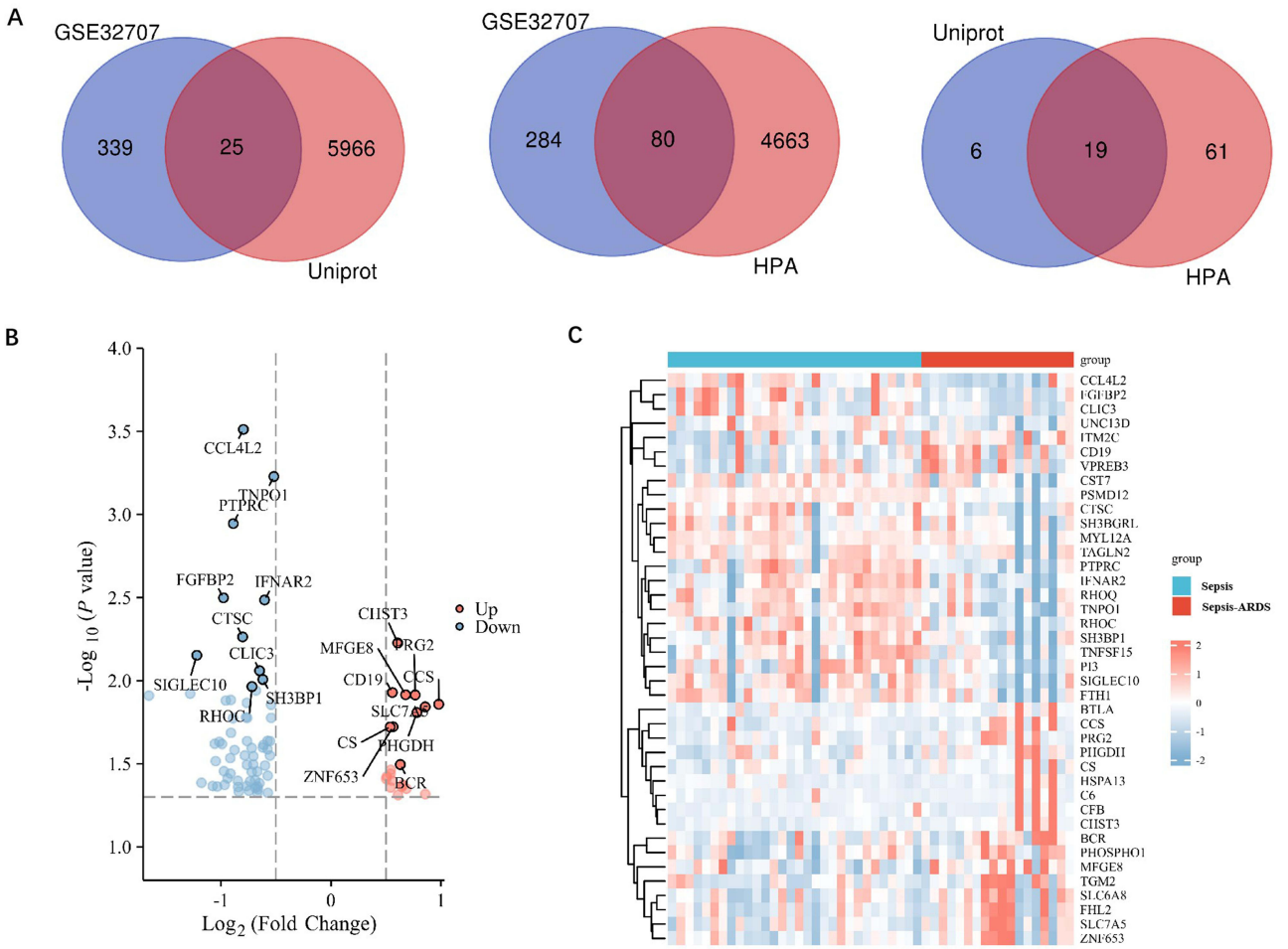

Figure 3 Screening of EP-DEGs. (**A**) The genes encoding extracellular proteins annotated in the HPA and UniProt database were intersected with DEGs. The genes screened by the two methods were combined to obtain a total of 86 EP-DEGs. (**B**) Volcano map of EP-DEGs in sepsis group and sepsis-induced ARDS group. Mark the top 10 up- regulated and down-regulated genes with the smallest P-value. (**C**) Heatmap of the top 20 up-regulated and top 20 down-regulated EP-DEGs.

significantly enriched in the top 5 biological processes (BPs), cellular components (CCs), and molecular functions (MFs), based on the smallest P-values (Figure 4B–D).

Identification and Validation of Hub Genes

The STRING database was used to build a PPI network comprising 86 EP-DEGs, and the network was visualized using Cytoscape software version 3.9.1. The resulting PPI network included 59 nodes and 112 edges (Figure 5A). Functional modules were identified using the MCODE plug-in in Cytoscape, revealing a top-scoring module with a score of 6.667, which contained 10 genes and 30 edges (Figure 5B). Additionally, the top 10 hub genes were identified using the MCC method within the Cytohubba tool (Figure 5C). These top 10 hub genes were further validated using five topological analysis methods available in the CytoHubba plug-in of Cytoscape (Table 2). There were 5 hub genes in all 5 methods, namely GNLY, GZMK, CST7, PTPRC and CD19 (Figure 5D). The expressions of the 5 hub genes between the two groups were analyzed in both the training set (GSE32707) and the validation set (GSE66890). We found that the expressions of GNLY, GZMK, CST7 and PTPRC were lower in the sepsis-induced ARDS group, while the CD19 expression was significantly higher in sepsis-induced ARDS group than sepsis group (Figure 6A and C). To further understand the diagnostic efficacy of the hub genes, ROC curve analysis was used to evaluate the accuracy of diagnosis in these two sets. The AUCs of CD19 in the GSE32707 was 0.719 (Figure 6B). And the AUC of CD19 in the GSE66890 dataset was 0.732 (Figure 6D), indicating that CD19 has definite diagnostic values.

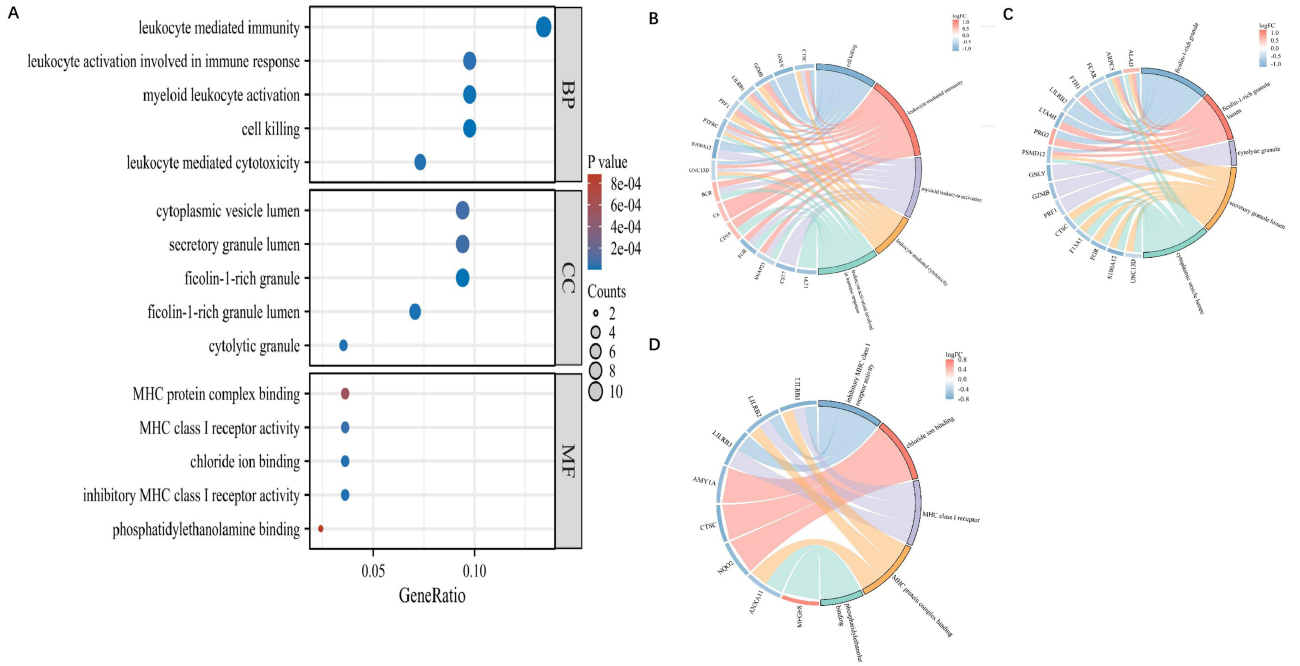

Figure 4 GO enrichment of EP-DEGs. (**A**) The dotplots show the top 5 processes enriched by EP-DEGs in BPs, CCs and MFs. (**B**–**D**) The circle graph shows the EP-DEGs enriched in the top 5 GO categories of BPs(**B**), CCs(**C**), and MFs(**D**). The size of the dots represents the number of enriched EP-DEGs while the color of the dots represents a p. adjust.
Abbreviations: BP, biological process; MF, molecular function; CC, cellular component.

Immune Cell Infiltration Analysis and Diagnostic Value of CD19$^+$ B Cell in Sepsis and Sepsis-Induced ARDS

To delve deeper into the potential roles of the peripheral blood immune cells in developing sepsis-induced ARDS, we examined the proportions of immune cells in sepsis and sepsis-induced ARDS patients of GSE32707 (Figure 7A). We investigated the differences in immune cell infiltration and found that B cells were significantly increased in sepsis-induced ARDS compared to sepsis controls (Figure 7B). To study whether CD19$^+$ B cells had any effect on human ARDS, we obtained the whole blood samples from patients on the day of admission. After adjusting for age differences through multiple linear regression, the sepsis-induced group exhibited significantly higher CD3-CD19+ counts (CD3-CD19+ Counts, β=28.87, 95% CI 15.04–42.7, p<0.001) and lower lymphocytes counts (Lymphocytes Count, β=−0.352, 95% CI −0.65–-0.05, p=0.022) compared to the sepsis group (Table 1 and Figure 8A). Moreover, CD19$^+$ B cell counts (AUC=0.829) had a higher accuracy in the diagnosis of septic-ARDS than other lymphocyte subsets, including killer T cells (CD3$^+$CD8$^+$), helper T cells (CD3$^+$CD4$^+$), and natural killer (NK) cells (CD3$^-$CD(16$^+$56)$^+$) (Figure 8B). All these studies showed that CD19$^+$ B cells may serve as a potential diagnostic role in sepsis induced-ARDS.

Discussion

ARDS is a heterogeneous syndrome, and its variability is primarily reflected in differences in physiology, imaging characteristics, underlying causes, timing of onset, biomarkers, and genetic factors, among other aspects.[17] As sepsis is the most common cause of ARDS, it is necessary to identify the potential diagnostic biomarkers of sepsis-induced ARDS.[2] In our study, we examined the gene expression profiles of whole blood samples obtained from patients with sepsis alone and those with septic ARDS in the training dataset (GSE32707). We identified 86 extracellular protein-related differentially expressed genes (EP-DEGs) and predicted the biological processes and pathways in which they are involved. Additionally, we pinpointed five hub genes—GNLY, GZMK, CST7, PTPRC, and CD19. The expression levels and diagnostic significance of CD19 were further validated using the validation dataset (GSE66890).

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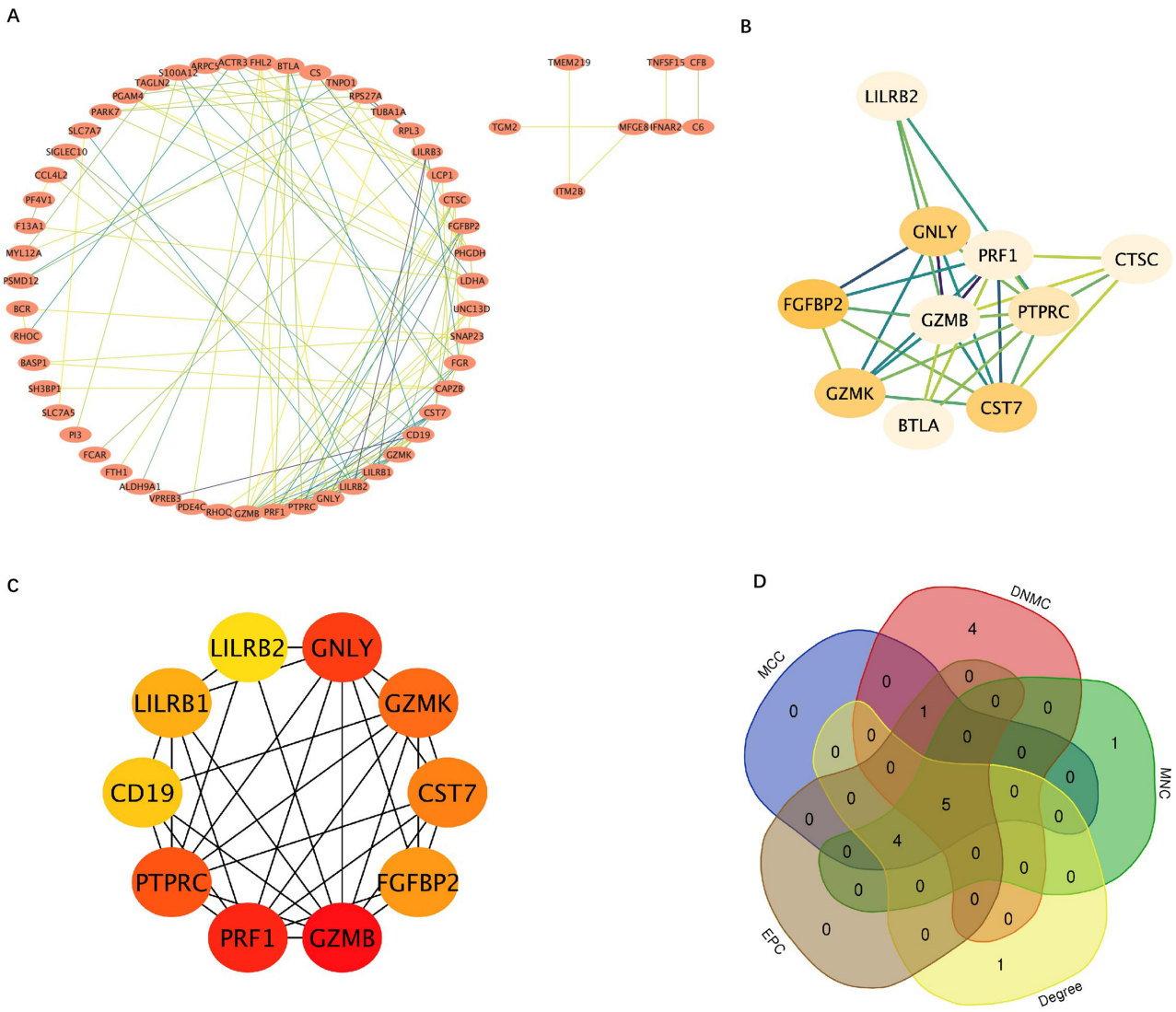

Figure 5 The construction of PPI network of EP-DEGs and screening of hub genes. (**A**) The STRING database is used to construct the PPI network of EP-DEGs. (**B**) The node gene cluster with the highest score constructed by the MCODE plug-in in Cytoscape. (**C**) The top 10 hub genes constructed by the MCC method of Cytohubba. (**D**) Venn Diagram showed the hub genes constructed by 5 topological methods of the CytoHubba plug-in in Cytoscape.

Utilizing the CIBERSORT algorithm, we analyzed differences in peripheral blood immune cell composition and observed an increase in B cells among septic-ARDS patients compared to those with sepsis alone. Furthermore, we confirmed the expression levels and diagnostic potential of $CD19^{+}$ B cells in clinical samples. Both bioinformatics

Table 2 Top10 EP-DEGs by 5 Topological Analysis Methods of CytoHubba

MCC	DNMC	MNC	Degree	EPC
GZMB	FGFBP2	GZMB	GZMB	PTPRC
PRF1	CST7	PRF1	PTPRC	GZMB
GNLY	GZMK	PTPRC	PRF1	PRF1
GZMK	BLTA	GNLY	GNLY	GNLY
CST7	CTSC	LILRB1	CD19	CST7
PTPRC	PTPRC	CD19	LILRB2	GZMK
FGFBP2	GNLY	LILRB2	LILRB1	LILRB1
LILRB1	CD19	CST7	GZMK	CD19
CD19	UNC13D	GZMK	CST7	LILRB2
LILRB2	CS	FGR	CAPZB	FGFBP2

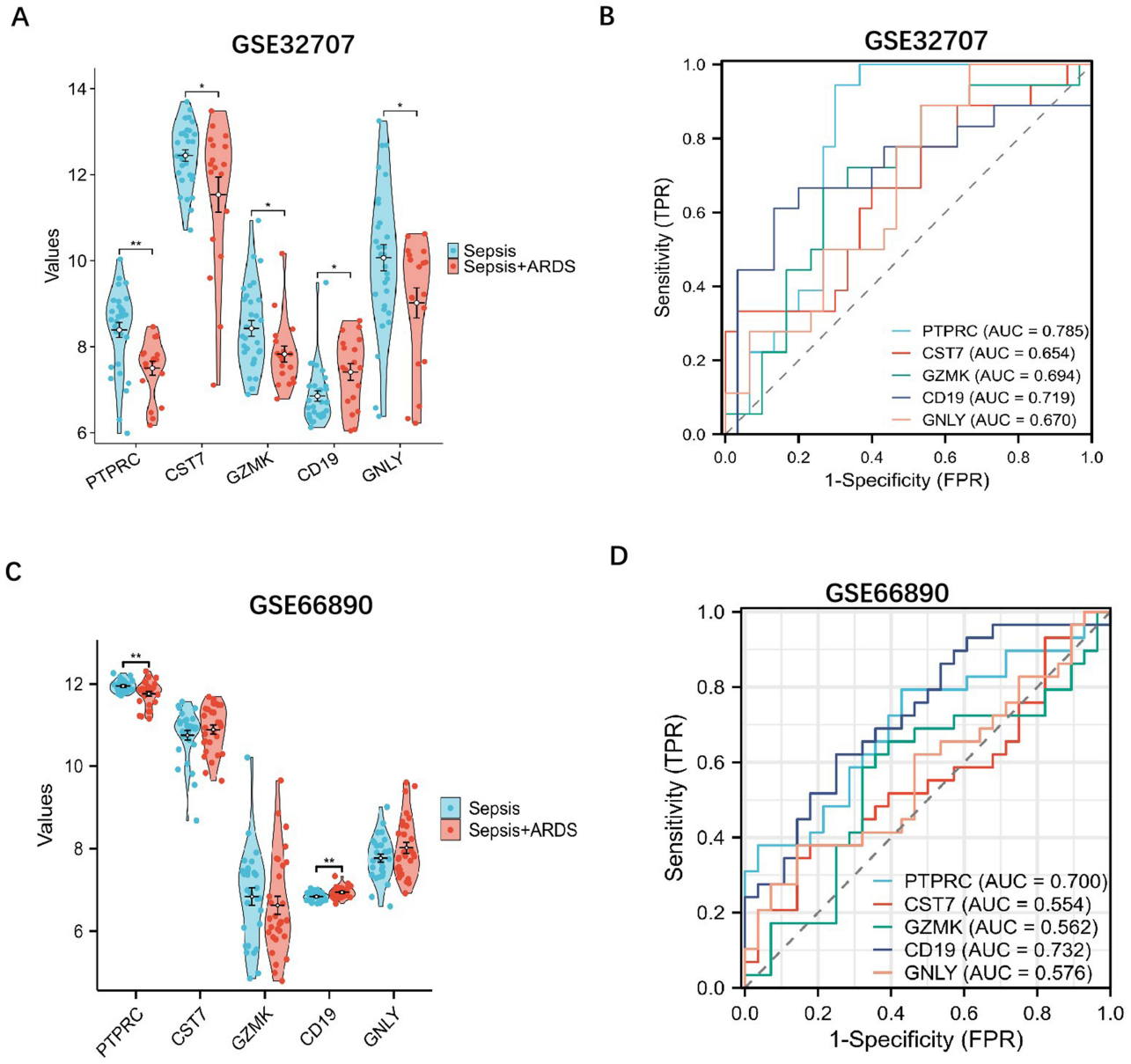

Figure 6 The expressions and diagnostic efficacy of the hub genes. (**A** and **C**) Differences in the expression of the hub genes between the two groups in training (GSE32707) and validation set (GSE66890). (**B** and **D**) The ROC curve analysis of the hub genes. *p < 0.05, **p < 0.01.

and clinical evidence suggest that $CD19^{+}$ B cells in peripheral blood may serve as potential diagnostic biomarkers for sepsis-induced ARDS.

We analyzed the GSE32707 dataset and identified a total of 364 DEGs. By cross-referencing these DEGs with extracellular protein gene lists from the UniProt and HPA protein annotation databases, we screened 86 EP-DEGs. 19 of the 86 EP-DEGs selected from the HPA database overlapped with those from UniProt, while only 6 genes did not overlap, demonstrating a high level of consistency between the two databases in annotating extracellular proteins. GO enrichment analysis revealed that the EP-DEGs were primarily enriched in processes such as leukocyte-mediated immunity, leukocyte activation involved in immune response, and myeloid leukocyte activation. These findings suggest that extracellular proteins may play a significant role in leukocyte-mediated immunity and contribute to the pathological mechanisms underlying sepsis-induced ARDS. The development of sepsis-induced ARDS was closely related to our immune system.[18] Neutrophils, acting as key effector cells in acute lung injury (ALI), are swiftly mobilized to the lungs

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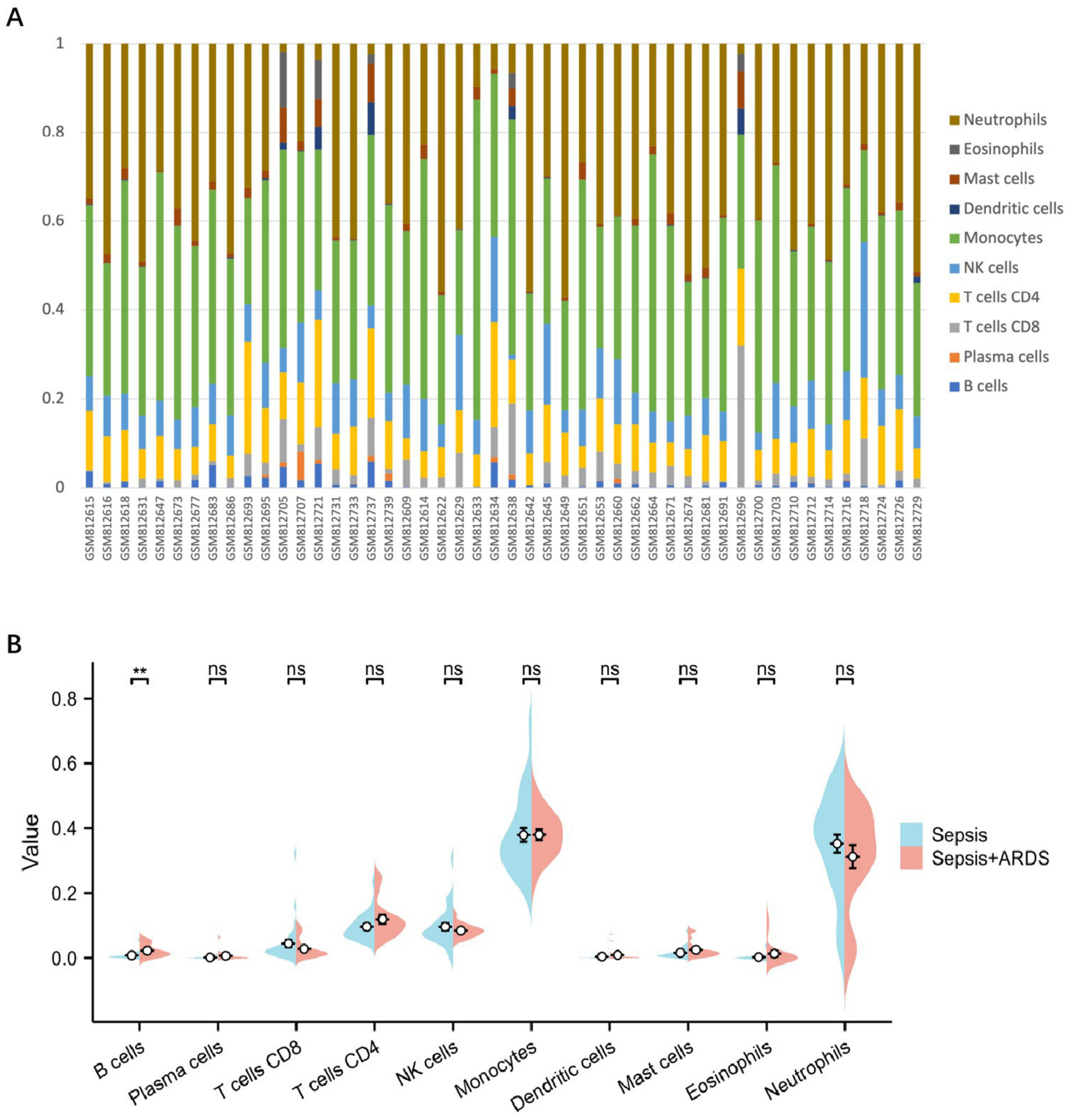

Figure 7 Landscape of immune infiltration between sepsis-induced ARDS and sepsis. (**A**) Bar charts of 10 immune cell proportions in sepsis-induced ARDS and sepsis groups. (**B**) Differential expression of different types of immune cell marker expression between sepsis-induced ARDS and sepsis groups. **p < 0.01.

following pathogen invasion.[19] During this process, pathogens trigger lung macrophages and epithelial cells to release chemokines, including TNF-α, IL-6, CXCL-1, CCL-2, GPR-35 (G-protein-coupled receptor), and MIP-2 (macrophage inflammatory protein-2), which facilitate the recruitment of neutrophils to the site of infection.[20,21] Neutrophils release a variety of destructive agents, including serine proteases, matrix metal-loproteinases, and lactoferrin, contributing to inflammation and damage lung tissue. Studies also reported that macrophage polarization plays important role in the immune mechanism of sepsis-induced ARDS.[22,23] Recent studies demonstrated that Th17/Treg ratio is higher in the peripheral blood of ARDS patients and associates with more adverse outcomes in ARDS patients.[24] Although the proinflammatory cytokines, such as TNF-a, IL-1b, IL-6, IL-8, and IL-18, are currently being actively researched in

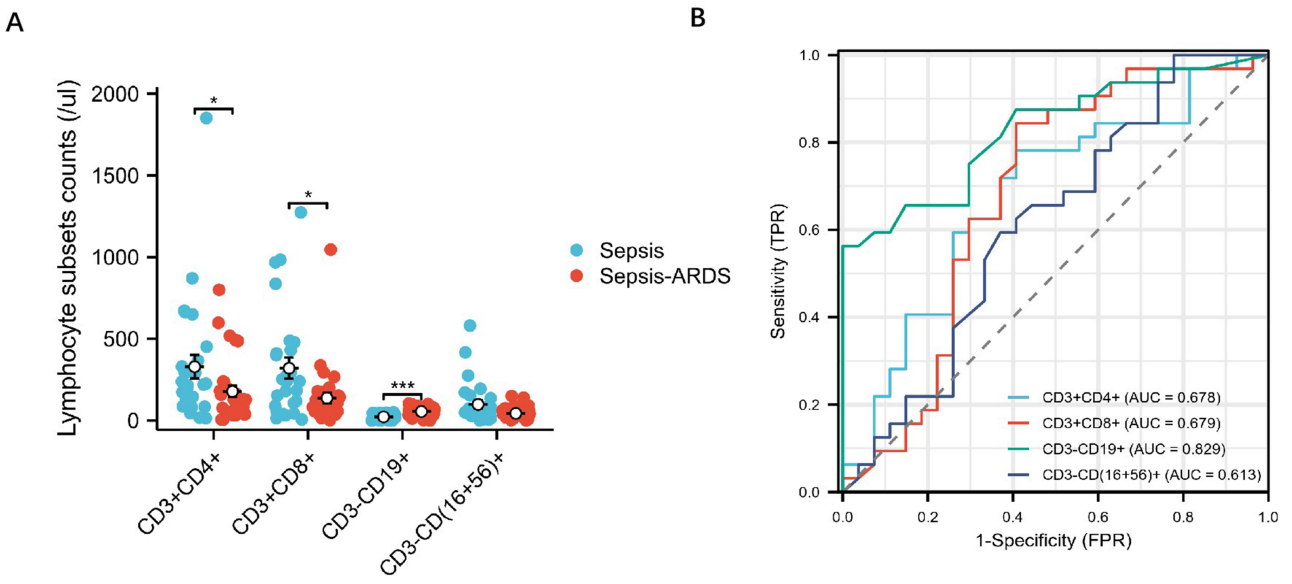

Figure 8 Lymphocyte subsets analysis in clinical samples. (**A**) The counts of lymphocyte subsets in two groups. (**B**) The ROC curve analysis of the lymphocyte subsets in two groups. *p < 0.05, ***p < 0.001.

animal studies; however, the diagnostic utility of these potential biomarkers in sepsis-induced ARDS remains insufficient in clinic.

The GO enrichment circle map shows that BCR, C6, and CD19 were enriched in multiple biological processes, including leukocyte-mediated immunity and immune response-related leukocyte activation. These genes might play a more important role in sepsis-induced ARDS. B cells express the co-receptor CD19, which serves as a positive regulator of B cell receptor (BCR) signaling and is critical for B cell development and activation.[25] As a pan-B-cell surface marker, CD19 is constitutively expressed on the surface of nearly all B-lymphocytes. These B cells serve as pivotal regulators of leukocyte activation processes, with emerging evidence underscoring their crucial involvement in the pathophysiological mechanisms underlying ARDS.[26,27] The infiltration levels of immune cells also showed that B cells were increased in septic-ARDS patients compared to sepsis alone patients. To further identify the potential diagnostic biomarkers in sepsis-induced ARDS, lymphocyte subsets were analyzed in the peripheral blood samples of clinical samples (27 sepsis and 32 sepsis-induced ARDS patients). Consistent with the bioinformatics results, analysis of clinical samples suggests that $CD3^{-}CD19^{+}$B cell counts were elevated and had a higher accuracy in the diagnosis of sepsis-induced ARDS group than other lymphocyte subsets. In this study, although the clinical validation sample size is small, based on the observed effect sizes in our study (eg, $CD3^{-}CD19^{+}$ Counts from Figure 8), the post-hoc power for detecting a Cohen's d=1.33 (large effect) with n=27 vs 32 reached 99% (α=0.05), far exceeding the conventional threshold of 80% and indicating sufficient statistical power for our primary findings. Increasing evidences are available regarding the role of B cell lymphocytes in the pathophysiology of ARDS. B cells are classified into B1 cells (B-1a and B-1b subsets), B2 cells (follicular cells and marginal zone cells), as well as regulatory B cells.[28] Researchers identified that B-1a cells might mitigate the inflammation and injury to the lungs in sepsis-induced ALI by animal experiments.[29] Regulatory B cells (Bregs) play a role in modulating immune responses, mainly through the secretion of the anti-inflammatory cytokine IL-10. Recent finding suggested that B cell-derived IL-10 alleviates lung inflammation and promotes recovery from LPS-induced ALI.[30] A recent study revealed that IgM+ peripheral B cells (T2B cells) selectively accumulate in lung capillaries through interactions involving CD49e and C-X-C motif chemokine receptor 5 (CXCR5). These cells help alleviate neutrophil-mediated inflammation by producing lipoxin A4 (LXA4).[27] Interestingly, the neutrophil-to-lymphocyte ratio (NLR) has emerged as a clinically significant biomarker for various lung diseases. Patients with both conventional ARDS and COVID-19–related ARDS tend to experience poorer outcomes when they exhibit an elevated NLR, particularly when this increase is driven by lymphopenia, which includes a reduction in

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peripheral circulating B cells.[31,32] These findings suggested the potential protective role of pan-B cells in sepsis-induced ARDS, but further exploration of underlying molecular mechanism of B cell subpopulations is warranted.

We recognize that there are several limitations in the present study. Firstly, the clinical sample size in this study was small (n = 27 vs 32). Although our cohort demonstrated adequate statistical power for core outcome assessments, definitive characterization of nuanced biological relationships and subgroup-specific interactions will require expanded sample sizes in subsequent validation studies. Secondly, our study only presents the potential diagnostic role of $CD19^+$ B cells in sepsis-induced ARDS, and does not demonstrate the causality or consequence in cell or animal models. Lastly, our current study primarily showed pan-B cells functions in ARDS pathogenesis, and consequently, the granular analysis of specific subsets falls beyond our present research. Despite these limitations, our results also provide valuable insights that could be applied to clinical practice.

In conclusion, our study suggests that peripheral $CD3^-CD19^+$ B cells could function as potential biomarkers for sepsis-induced ARDS. This finding may provide a new strategy for the diagnosis of this complex condition.

Data Sharing Statement

The datasets generated and/or analyzed during this study are available from the corresponding author upon reasonable request.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of Qilu Hospital, Shandong University (Approval No. KYLL-202202-027-1). All procedures were conducted in compliance with relevant guidelines and regulations. Written informed consent was obtained from all patients and legal guardians of pediatric patients. This study was performed following the Declaration of Helsinki.

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Author Contributions

All authors contributed to data analysis, drafting or revising the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no conflicts of interest in this work.

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