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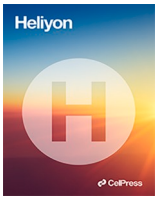

Research article

Data-driven analysis that integrates bioinformatics and machine learning uncovers PANoptosis-related diagnostic genes in early pediatric septic shock

Jing Wang [1], ShiFeng Chen [1], Lei Chen [1], Dajie Zhou [*]

Department of Clinical Laboratory Center, Yantai Yuhuangding Hospital, Yantai, 264099, Shandong, China

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ABSTRACT

Objectives: Sepsis is one of the leading causes of death for children worldwide. Additionally, refractory septic shock is one of the most significant groups that contributes to a high death rate. The interaction of pyroptosis, apoptosis, and necroptosis results in a unique inflammatory cell death mechanism known as PANoptosis. An increasing amount of evidence suggests that PANoptosis can be brought on by several stimuli, including cytokine storms, malignancy, and bacterial or viral infections. The goal of this study is to improve the diagnostic significance of the PANoptosis-related gene signature in early pediatric septic shock.

Design and methods: We examined children with septic shock from the GSE66099 discovery cohort and looked at differentially expressed genes (DEGs). To filter the important modules, weighted gene co-expression network analysis (WCGNA) was employed. In the end, random forest analysis and the least absolute shrinkage and selection operator (LASSO) were used to determine the PANoptosis diagnostic signature genes. To determine the PANoptosis signature genes, we also found four validation cohorts: GSE26378, GSE26440, GSE8121, and GSE13904. The area under the curve (AUC) of the receiver operating characteristic curves (ROCs), along with sensitivity, specificity, positive predictive value, and negative predictive value, were used to assess the diagnostic efficacy of these signature genes.

Results: From GSE66099, 1142 DEGs in total were tested. Following the WGCNA clustering of the data into 16 modules, the MEgrey module showed a significant correlation with pediatric septic shock ($p < 0.0001$). Following the use of LASSO and random forest algorithms to identify the PANoptosis-related signature genes, which include ANXA3, S100A9, TXN, CLEC5A, and TMEM263. These signature genes' receiver operating characteristic curves (ROCs) were confirmed in the external dataset from GSE26378, GSE26440, GSE8121, and GSE13904, and were 0.994 (95 % CI 0.987–0.999), 0.987 (95 % CI 0.974–0.997), 0.957 (95 % CI 0.927–0.981), 0.974 (95 % CI 0.954–0.988), 0.897 (95 % CI 0.846–0.941), respectively.

Conclusion: In summary, the discovery of PANoptosis genes, ANXA3, S100A9, TXN, CLEC5A, and TMEM263 proved to be quite helpful in the early detection of pediatric septic shock patients. These early results, which need to be further confirmed in basic and clinical research, are extremely important for understanding immune cell infiltration in the pathophysiology of pediatric septic shock.

* Corresponding author. Yantai, 264099, Shandong, China.
E-mail address: dajie71@126.com (D. Zhou).

[1] Jing Wang, ShiFeng Chen, and Lei Chen contributed equally to this work.

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1. Introduction

Globally, sepsis is one of the main causes of death for children [1,2]. Furthermore, the two most important categories that contribute to a high death rate in pediatric sepsis are refractory septic shock and multiple organ failure syndrome with a high mortality rate ranging from 40 to 80 % [3,4]. According to an emergency department study, hospital mortality rates for septic shock and sepsis were 8.0 % and 0.9 %, respectively [5,6]. There are an estimated 2202 occurrences of neonatal sepsis per 100,000 live births and 22 cases of pediatric sepsis per 100,000 person-years worldwide, which translates to 1.2 million cases of childhood sepsis annually [7]. Refractory shock and/or multiple organ failure syndrome are the main causes of death for children with sepsis, and many of these deaths happen within the first 48–72 h of treatment [8]. Controlling infection, correcting hemodynamic irregularities, ensuring vital organ perfusion, stabilizing the airway, and facilitating appropriate breathing with oxygen supply are all critical components of treating septic shock [9,10]. Therefore, early diagnosis is necessary to improve the prognosis and reduce mortality in children with septic shock.

A novel inflammatory cell death mechanism called PANoptosis is a result of the interplay between pyroptosis, apoptosis, and necroptosis. A growing body of research indicates that a variety of triggers, such as cytokine storms, cancer, and bacterial or viral infections, can cause PANoptosis [11]. Theoretically, PANoptosis seems to defend against the majority of acute bacterial pathogens that infect hosts and, frequently, even more successfully limits opportunistic or non-pathogenic microorganisms [12,13]. Furthermore, an increasing number of studies have demonstrated that novel PANoptosis biomarkers have the potential to be targets for disease immunotherapy in addition to being predictive of diseases, particularly infectious diseases [14–16].

In this work, we investigated potential gene biomarkers for early pediatric septic shock by combining bioinformatics analysis with machine learning-based mining of publicly available databases. First, in both the healthy children and the early pediatric septic shock patients, we methodically determined which genes associated with PANoptosis were differentially expressed. We then discovered that 27 PANoptosis genes that were differently expressed were potential genes that are most relevant to pediatric septic shock. LASSO and random forest eventually assist in identifying the diagnostic genes for children with septic shock by focusing on these 27 specific genes. ANXA3, S100A9, TXN, CLEC5A, and TMEM263 are the five early biomarkers of septic shock in children that we have finally identified. By constructing receiver operating characteristic (ROC) curves and calculating the related areas under the curve (AUCs) in an external dataset, we have also confirmed the diagnostic efficacy of these biomarkers. All in all, we discovered five genes that may have significant diagnostic implications in pediatric septic shock, offering theoretical support and a theoretical framework for the early diagnosis of pediatric septic shock.

2. Methods

2.1. Datasets and PANoptosis genes acquisition

Five datasets, including GSE66099, GSE26378, GSE26440, GSE8121, and GSE13904, have been retrieved from Gene Expression Omnibus (GEO) for the current investigation. 228 cases, comprising 47 normal controls and 181 children in septic shock, made up the discovery set GSE66099. As validation sets, GSE26378, GSE26440, GSE8121, and GSE13904 were employed (Table 1). There were 21 normal controls and 82 children in the validation set GSE26378 who were in septic shock. 32 normal controls and 98 children in septic shock made up the validation set GSE26440. 15 normal controls and 30 children in septic shock made up the validation set GSE8121. There were 18 normal controls and 106 children in the validation set GSE13904. The genes for pyroptosis and necroptosis were procured from Genecard (https://www.genecards.org/), whilst the genes for apoptosis were sourced from MSigDB HALLMARK-APOPTOSIS.v2023.2. Hs. A total of 1223 PANoptosis genes were obtained after deleting duplicates (Supplement Table 1).

2.2. Acquiring of candidate differentially expressed genes

Differentially expressed genes (DEGs) between the pediatric septic shock cohort and control cohort were evaluated using the limma package in R software, using the following criteria: P value should be adjusted to <0.05 and $|\log \text{fold change (FC)}| > 1$ [17]. Finding genes with varying expression levels between the septic shock group and the healthy group is the aim of differential analysis. Second, looking for PANoptosis genes with differential expression can help identify possible diagnostic markers.

Table 1
The datasets employed in our study.

Dataset	Study patients were included	Time	Experiment type	Platforms
GSE66099	47 Healthy Control vs 181 Pediatric Septic Shock	24h	Expression profiling by array	GPL570
GSE26378	21 Healthy Control vs 82 Pediatric Septic Shock	24h	Expression profiling by array	GPL570
GSE26440	32 Healthy Control vs 98 Pediatric Septic Shock	24h	Expression profiling by array	GPL570
GSE8121	15 Healthy Control vs 30 Pediatric Septic Shock	24h	Expression profiling by array	GPL570
GSE13904	18 Healthy Control vs 106 Pediatric Septic Shock	72h	Expression profiling by array	GPL570

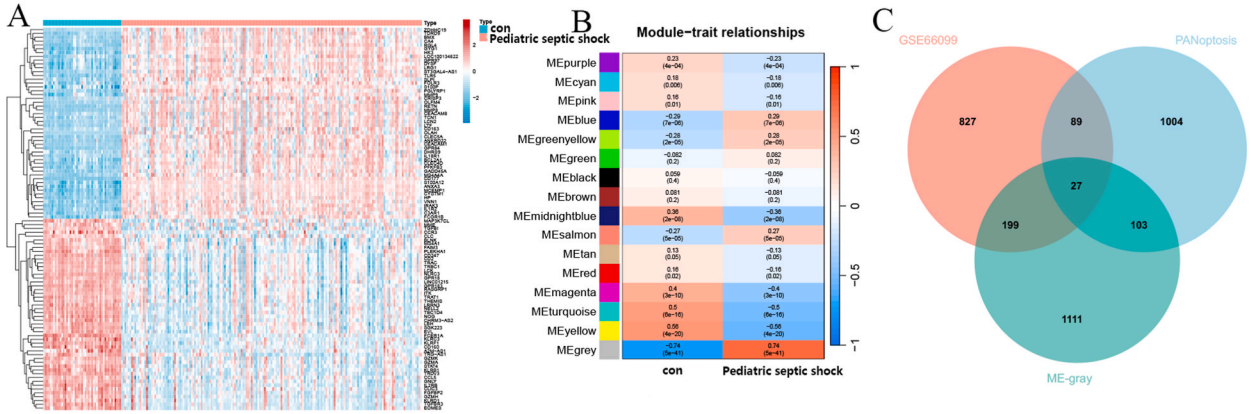

Fig. 1. Identification of PANoptosis-associated differential genes for pediatric sepsis shock. (A) Heatmap of the top 50 up- and down-regulated DEGs between pediatric sepsis shock and healthy control in the GSE66099 cohort. (B) Trait and module correlation of WGCNA. A heatmap illustrating the correlation between pediatric septic shock features and modules. The value in each cell indicates the correlation score, while the value in the bracket below indicates the significance (P-value). (C) The veen plot showed the interaction between DEGs, MEgrey module, and PANoptosis. ($P < 0.05$).

2.3. Weighted gene Co-expression network analyses

The expression profiling from the GSE66099 datasets was obtained using the WGCNA package to carry out Weighted Gene Co-expression Network Analysis [18]. The adjacency matrix was used to build the topological overlap matrix (TOM). Various co-expression modules were grouped based on the dissimilarity measured by TOM. To establish a connection between modules and pediatric septic shock, estimates of module membership (MM) and gene significance (GS) were made, and ultimately identified the key modules. The purpose of WGCNA is to identify the gene modules most strongly associated with septic shock in children.

2.4. Identification and validation of PANoptosis-related diagnostic biomarkers for pediatric septic shock

Genes shared by DEGs, WGCNA, and PANoptosis could be promising candidates for pediatric septic shock diagnostic biomarkers, according to GSE66099 datasets. Candidate genes were then screened using two machine learning algorithms: random forest and least absolute shrinkage and selection operator (LASSO). The random forest technique was applied in this work using the "randomForest" R package in R. Genes that have a significance score higher than 2 was chosen. With the "glmnet" R package and penalty parameters for 10-fold cross-validation, LASSO analysis was carried out. This method is better than logistic regression analysis for analyzing high-dimensional data, and minimal lambda was found to be ideal. Children suffering from septic shock had signature genes that coincided with the intersection genes of these two machine learning techniques. The diagnostic effectiveness of these signature genes was evaluated using the area under the curve (AUC) of the receiver operating characteristic curves (ROCs), as well as Sensitivity, Specificity, Positive Predictive Value, and Negative Predictive Value. In both the validation sets (GSE26378, GSE26440, GSE8121, and GSE13904) and the discovery set (GSE66099), every diagnostic gene expression was computed.

2.5. Functional enrichment analysis of 89 overlap PANoptosis genes

Functional enrichment analyses of differentially expressed PANoptosis genes based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) were performed using the clusterProfiler package in R [19]. Three categories—biological process (BP), cellular component (CC), and molecular function (MF)—were found as part of the GO analysis, and these categories helped to shed light on the biological processes of these DEGs. Utilizing KEGG analysis, possible signaling pathways were investigated. This allows for the identification of PANoptosis gene functions and their participation in biological processes in septic shock children.

2.6. Examination of immune cell infiltration

For this experiment, we employed CIBERSORT to acquire gene expression matrices of 22 tumor-infiltrating immune cells (TIICs) from healthy and pediatric septic shock groups. Next, between children with septic shock and healthy, we obtained significantly different immune infiltrating cells.

2.7. Statistical analysis

R software (version 4.2.2) was used to conduct all statistical analyses in this study. With a p-value of less than 0.05, a two-tailed test was used to evaluate statistical significance.

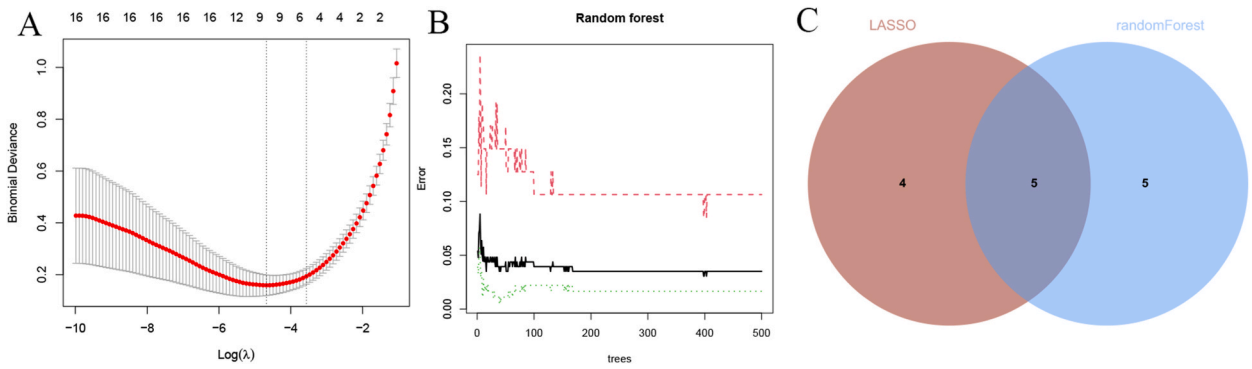

Fig. 2. Developing a PANoptosis diagnostic signature evaluation for pediatric septic shock. (A) Using Lasso regression analysis and 10-fold cross-validation, nine PANoptosis genes linked to pediatric septic shock were identified. (B) Ten PANoptosis genes associated with pediatric septic shock were found by random forest model analysis. (C) The five PANoptosis diagnostic signatures shared between LASSO and the random forest model were displayed in the veen plot.

3. Results

3.1. Determining the DEGs in septic shock and control among children

Using the "limma" program, DEGs from kids in septic shock and healthy controls were examined. After screening 1142 DEGs in total, 585 genes were found to be up-regulated and 557 genes to be down-regulated (Supplement Table 2). The top 50 down-regulated and top 50 up-regulated DEGs between the healthy group and the children with septic shock were displayed on the heatmap (Fig. 1A).

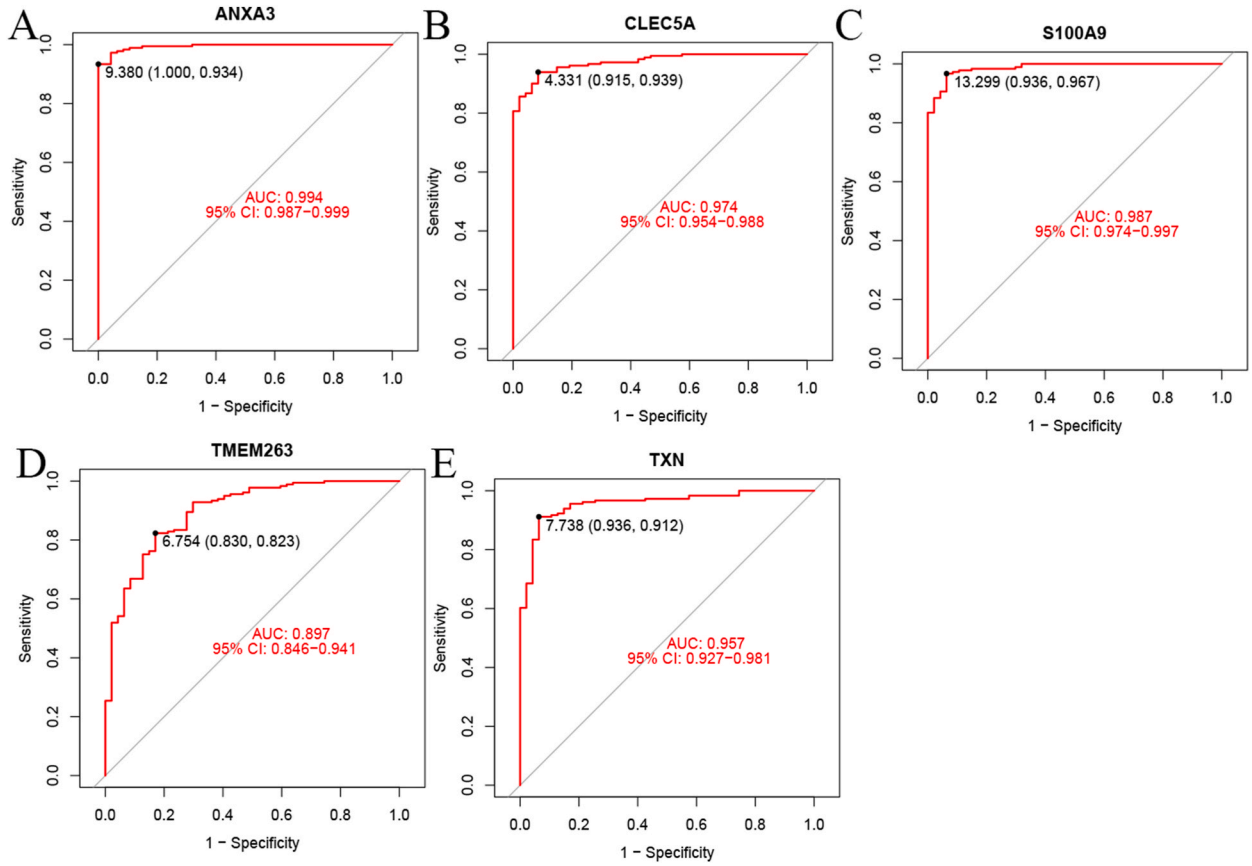

Fig. 3. The ROC curve was utilized in the discovery cohort GSE66099 to investigate the predictive capacity of five PANoptosis genes for pediatric septic shock. High AUC values and strong PPV, NPV, sensitivity, and specificity indicate strong predictive abilities. (A) ANXA3. (B) CLEC5A. (C) S100A9. (D) TMEM263. (E) TXN.

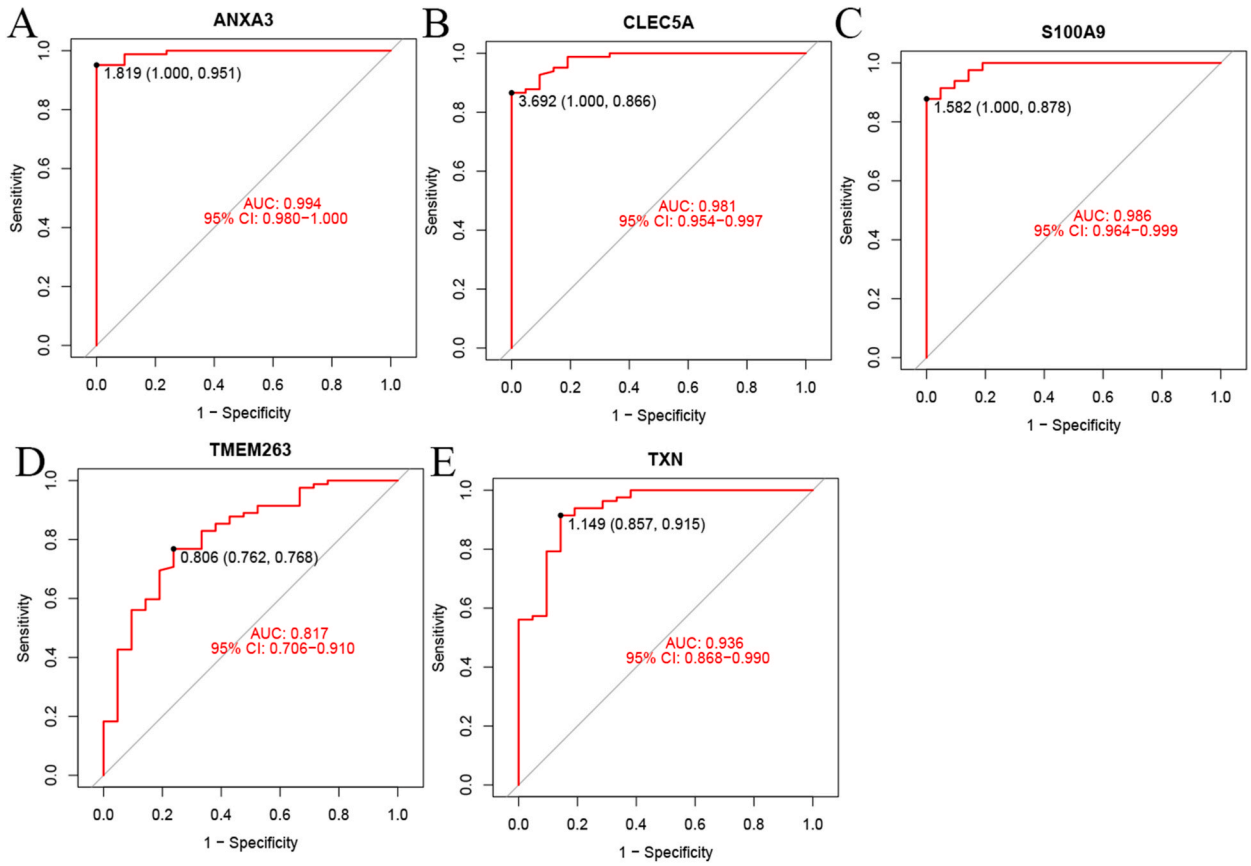

Fig. 4. The ROC curve was utilized in the validation cohort GSE26378 to investigate the predictive capacity of five PANoptosis genes for pediatric septic shock.(A) ANXA3. (B) CLEC5A. (C) S100A9. (D) TMEM263. (E) TXN.

3.2. Building the weighted gene co-expression network

Using the WGCNA package in R software, children suffering from septic shock as well as healthy individuals were studied, and a scale-free co-expression network was created. At last, 16 modules were created from the data cluster (Fig. 1B). Calculations were made to determine the correlation between each module and the normal and septic shock children. The MEgrey module was shown to be substantially associated with children experiencing septic shock (cor = 0.74, $p < 0.0001$), according to the data. As a result, the MEgrey module had 1440 genes, which were thought to be crucial for understanding infants who had sepsis shock. Fig. 1C displayed the 27 genes that overlapped between the DEGs, MEgrey module, and PANoptosis. These 27 genes may be good candidates as diagnostic biomarkers for pediatric septic shock (Supplement Table 3).

3.3. Signature gene selection using the LASSO and random forest techniques

In children suffering from septic shock, two machine algorithms were utilized to separate candidate 27 genes from PANoptosis genes. Nine signature genes were chosen for the LASSO analysis (Fig. 2A), while ten signature genes with significance scores greater than 2 were identified for the random forest analysis (Fig. 2B). ANXA3, S100A9, TXN, CLEC5A, and TMEM263 are the five pediatric septic shock signature genes that were ultimately identified by the combination of these two algorithms (Fig. 2C).

3.4. The ability of ANXA3, S100A9, TXN, CLEC5A, and TMEM263 signature genes to diagnose and predict pediatric septic shock

The area under the curve (AUC) values of ANXA3, S100A9, TNX, CLEC5A, and TMEM263 in the discovery cohort GSE66099 were 0.994, 0.987, 0.957, 0.974, 0.897, respectively (Fig. 3A–E). Furthermore, the AUC values of ANXA3, S100A9, TNX, CLEC5A, and TMEM263 in the validation cohort GSE26378 were 0.994, 0.986, 0.936, 0.981, 0.817 (Fig. 4A–E). The AUC values of ANXA3, S100A9, TNX, CLEC5A, and TMEM263 in the validation cohort GSE26440 were 0.989, 0.977, 0.936, 0.984, 0.893 (Fig. 5A–E). The AUC values of ANXA3, S100A9, TNX, CLEC5A, and TMEM263 in the validation cohort GSE8121 were 0.976, 0.947, 0.889, 0.958, 0.896 (Fig. 6A–E). The AUC values of ANXA3, S100A9, TNX, CLEC5A, and TMEM263 in the validation cohort GSE13904 were 0.960, 0.959,

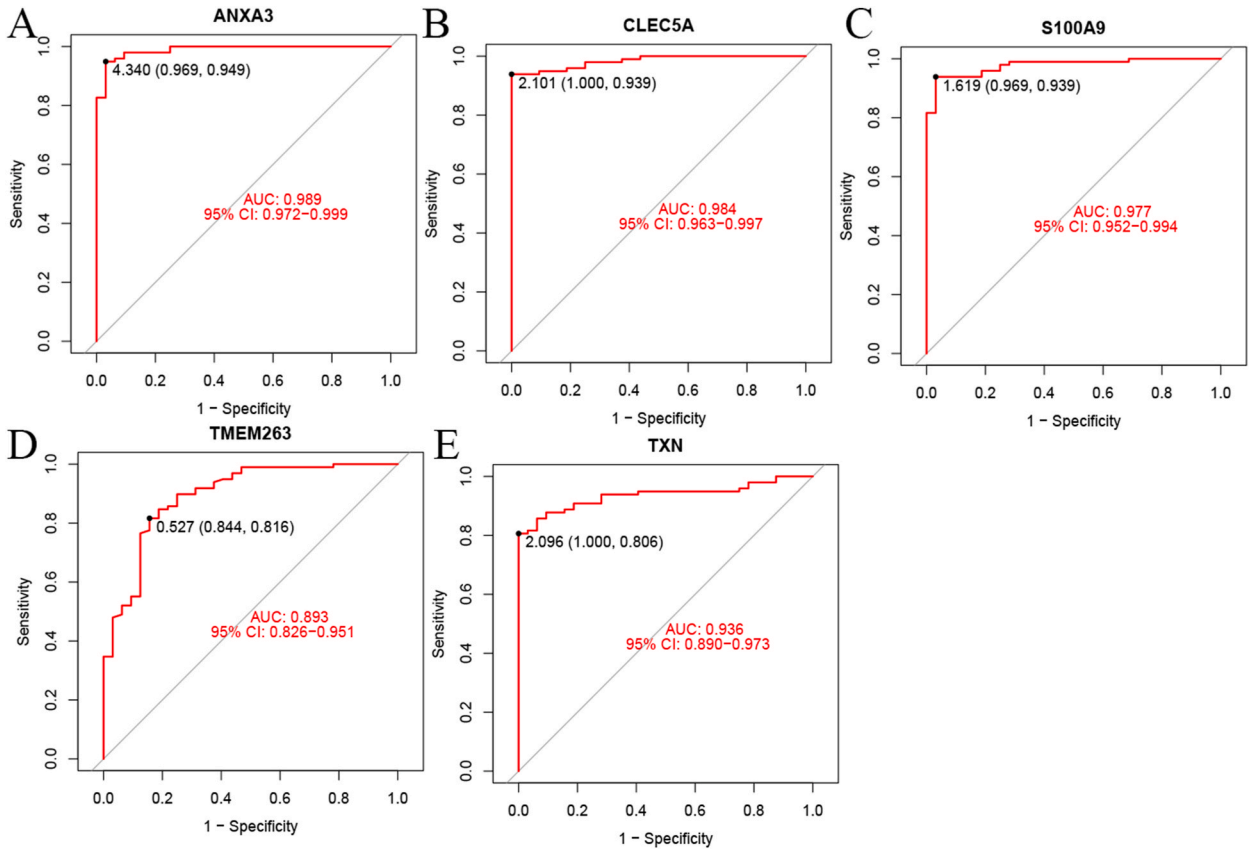

Fig. 5. The ROC curve was utilized in the validation cohort GSE26440 to investigate the predictive capacity of five PANoptosis genes for pediatric septic shock.(A) ANXA3. (B) CLEC5A. (C) S100A9. (D) TMEM263. (E) TXN.

0.907, 0.962, 0.878 (Fig. 7A–E). Table 2 displayed the specifics of AUC, confidence intervals, sensitivity, specificity, positive predictive value, and negative predictive value. The findings show that ANXA3, S100A9, TNX, CLEC5A, and TMEM263 both have high diagnostic values.

3.5. Differentially expression of ANXA3, S100A9, TXN, CLEC5A, and TMEM263 signature genes in healthy and pediatric septic shock

The screened signature genes of ANXA3, S100A9, TXN, and CLEC5A were highly expressed in children with sepsis shock compared to those in healthy children in the discovery cohort GSE66099 and validation cohorts GSE26378, GSE26440, GSE8121, and GSE13904. Yet, in the discovery and validation cohorts, children with septic shock had lower levels of TMEM263 expression than healthy children. This suggests that these genes may have a role in pediatric sepsis shock (Fig. 8A–E, $p<0.001$).

3.6. Functional enrichment analysis of 89 differentially expressed PANoptosis genes

To better understand the biological roles of the PANoptosis genes in pediatric septic shock, we performed functional analysis. Supplement Table 3 (column GSE66099|PANoptosis) displayed all 89 differentially expressed PANoptosis genes. The three categories that make up the GO analysis are BP, CC, and MF (Fig. 9A). Regulation of inflammatory response, positive regulation of cytokine production, positive regulation of NF-kappaB transcription factor activity, response to lipopolysaccharide, response to molecule of bacterial origin, and pyroptosis were the key areas of enrichment for the top six BP. The top six CC were primarily enriched in secretory granule lumen, cytoplasmic vesicle lumen, vesicle lumen, specific granule lumen, inflammasome complex, and collagen-containing extracellular matrix. Additionally, the top six MF were primarily enriched in protease binding, endopeptidase activity, pattern recognition receptor activity, serine-type endopeptidase activity, lipopolysaccharide-binding, serine-type peptidase activity (adj. $p < 0.05$). As shown in the KEGG analysis, the top six enriched pathways were mainly NOD-like receptor signaling pathway, Salmonella infection, Legionellosis, NF-kappa B signaling pathway, TNF signaling pathway as well as tuberculosis (adj. $p < 0.05$) (Fig. 9B).

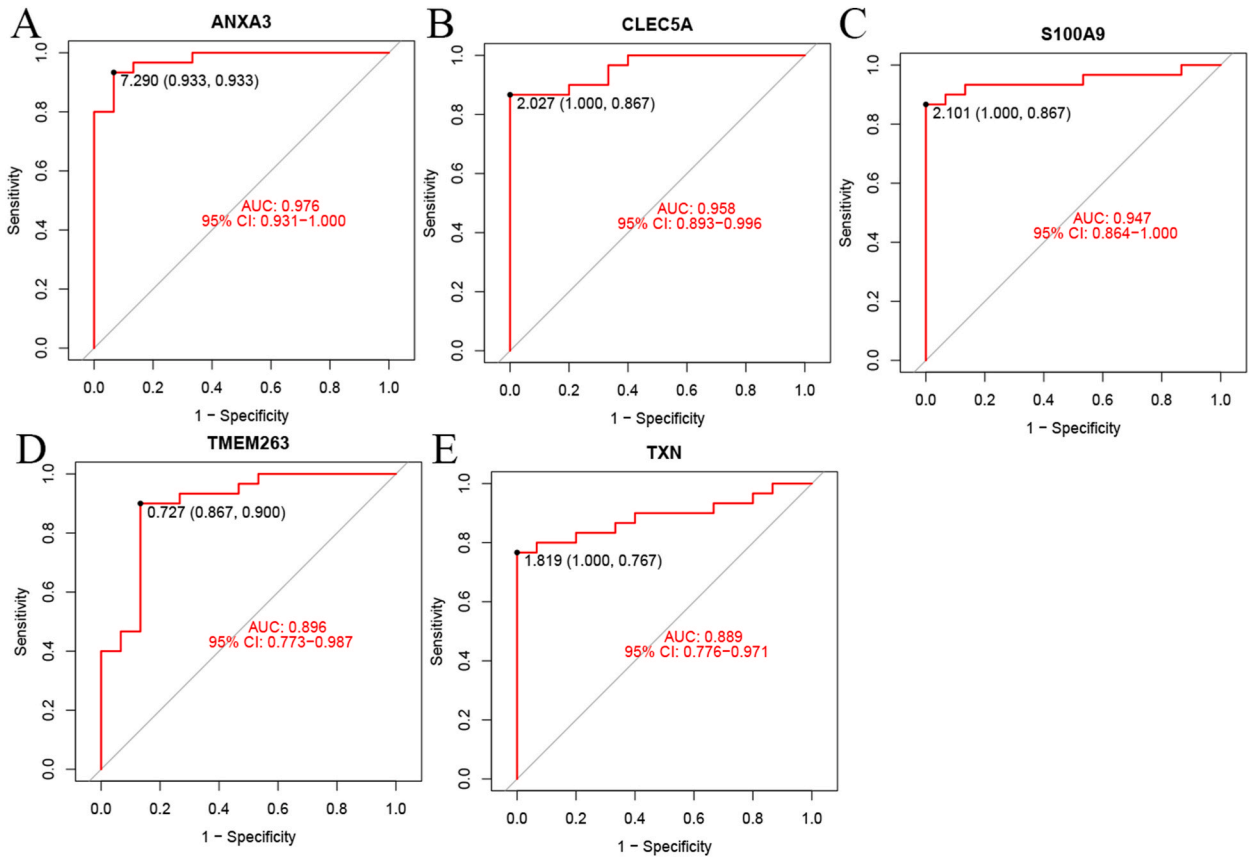

Fig. 6. The ROC curve was utilized in the validation cohort GSE8121 **to investigate the predictive capacity of five PANoptosis genes for pediatric septic shock.**(A) ANXA3. (B) CLEC5A. (C) S100A9. (D) TMEM263. (E) TXN.

3.7. Infiltration of immune cells results

We plotted a heatmap to demonstrate the proportion of 22 immune cells in each healthy and pediatric septic shock sample (Fig. 10A). Pediatric sepsis shock samples, according to the CIBERSORT algorithm, typically had higher proportions of neutrophils, monocytes, Macrophages M0, Macrophages M1, T cells follicular helper, plasma cells, but lower proportions of B cells naive, T cells CD8, T cells CD4 naive, T cells CD4 memory resting, T cells CD4 memory activated, T cells gamma delta, NK cells resting, and Dendritic cells resting, respectively ($p < 0.05$) (Fig. 10B).

4. Discussion

Antimicrobial medications must be taken empirically and promptly in cases of life-threatening infections. There is a higher chance of negative consequences and mortality when antibiotic treatment for septic shock in children is started later than is necessary. The relationship between early antibiotic therapy and lower fatality rates related to pediatric sepsis and septic shock was examined in two retrospective observational studies [20,21]. A potentially deadly multi-organ failure brought on by the body's dysregulated reaction to an infectious process is known as sepsis shock. This dysregulated reaction could start with an excessive and uncontrollably proinflammatory expression [22]. Refractory shock is the primary cause of death in children with sepsis and septic shock, accounting for one-third of deaths during the first 72 h [8,23]. Therefore, finding an early, specific diagnostic marker is crucial to improving the outcome of pediatric sepsis shock patients. Hence, whole blood RNA sample expression data from children experiencing septic shock during the first 24 h (GSE66099, GSE26378, GSE26440, GSE8121) and 72 h (GSE13904) are included in our study cohort [24–28].

This study investigated the essential components of WGCNA-based sepsis shock in pediatric patients and assessed the DEGs between pediatric septic shock and normal populations. Next, take the PANoptosis intersection gene. As a potential biomarker of PANopotosis in pediatric septic shock. Using both LASSO and random forest analysis, the signature genes for pediatric septic shock were found to include ANXA3, S100A9, TXN, CLEC5A, and TMEM263. After that, we used four external tests to confirm these gene markers. These five biomarkers exhibit strong positive predictive values, sensitivity, and specificity.

In total, 1142 DEGs were found between the pediatric sepsis shock and the healthy groups, with 585 genes being upregulated and 557 genes being downregulated. We identified 89 differentially expressed PANoptosis genes to assess the functional enrichment of

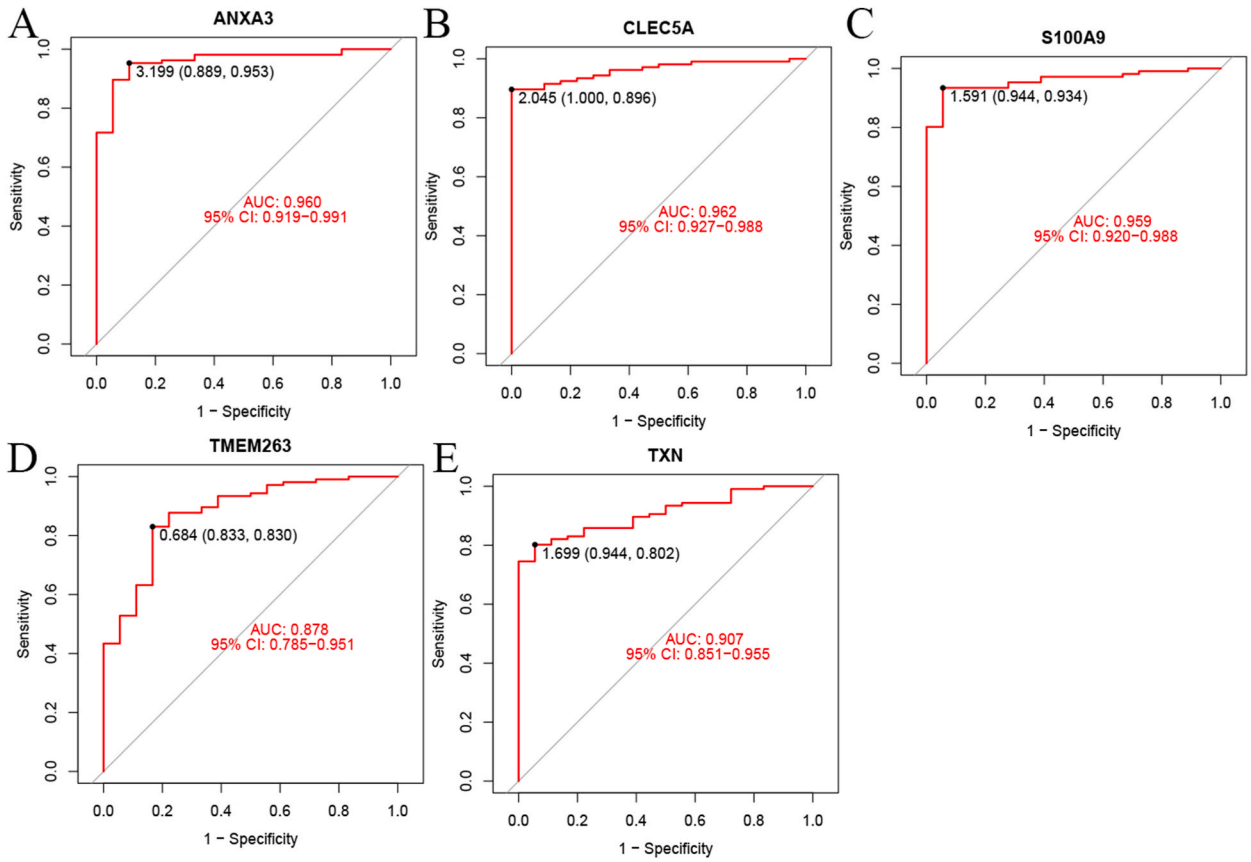

Fig. 7. The ROC curve was utilized in the validation cohort GSE13904 to investigate the predictive capacity of five PANoptosis genes for pediatric septic shock.(A) ANXA3. (B) CLEC5A. (C) S100A9. (D) TMEM263. (E) TXN.

Table 2
The diagnostic effectiveness of these signature genes.

GEO dataset	Gene marker	AUC	Cutoff	sensitivity	specificity	PPV	NPV
GSE66099	ANXA3	0.994 (0.987–0.999)	9.379	0.933	1	1	0.796
	S100A9	0.987 (0.974–0.997)	13.298	0.966	0.936	0.983	0.88
	TXN	0.957 (0.927–0.981)	7.738	0.911	0.936	0.982	0.733
	CLEC5A	0.974 (0.954–0.988)	4.331	0.939	0.914	0.977	0.796
	TMEM263	0.897 (0.846–0.941)	6.753	0.823	0.829	0.949	0.549
GSE26378	ANXA3	0.994 (0.980–1.000)	1.819	0.951	1	1	0.84
	S100A9	0.986 (0.964–0.999)	1.581	0.878	1	1	0.677
	TXN	0.936 (0.868–0.990)	1.149	0.914	0.857	0.961	0.72
	CLEC5A	0.981 (0.954–0.997)	3.691	0.865	1	1	0.656
	TMEM263	0.817 (0.706–0.910)	0.805	0.768	0.761	0.926	0.457
GSE26440	ANXA3	0.989 (0.972–0.999)	4.339	0.948	0.968	0.989	0.861
	S100A9	0.977 (0.952–0.994)	1.618	0.938	0.968	0.989	0.837
	TXN	0.936 (0.890–0.973)	2.096	0.806	1	1	0.627
	CLEC5A	0.984 (0.963–0.997)	2.101	0.938	1	1	0.842
	TMEM263	0.893 (0.826–0.951)	0.527	0.816	0.843	0.941	0.6
GSE8121	ANXA3	0.976 (0.931–1.000)	7.290	0.933	0.933	0.965	0.875
	S100A9	0.947 (0.864–1.000)	2.100	0.866	1	1	0.789
	TXN	0.889 (0.776–0.971)	1.819	0.766	1	1	0.681
	CLEC5A	0.958 (0.893–0.996)	2.027	0.866	1	1	0.789
	TMEM263	0.896 (0.773–0.987)	0.726	0.9	0.866	0.931	0.812
GSE13904	ANXA3	0.960 (0.919–0.991)	3.199	0.952	0.888	0.980	0.761
	S100A9	0.959 (0.920–0.988)	1.591	0.933	0.944	0.99	0.708
	TXN	0.907 (0.851–0.955)	1.698	0.801	0.944	0.988	0.447
	CLEC5A	0.962 (0.927–0.988)	2.044	0.896	1	1	0.620
	TMEM263	0.878 (0.785–0.951)	0.683	0.830	0.833	0.967	0.454

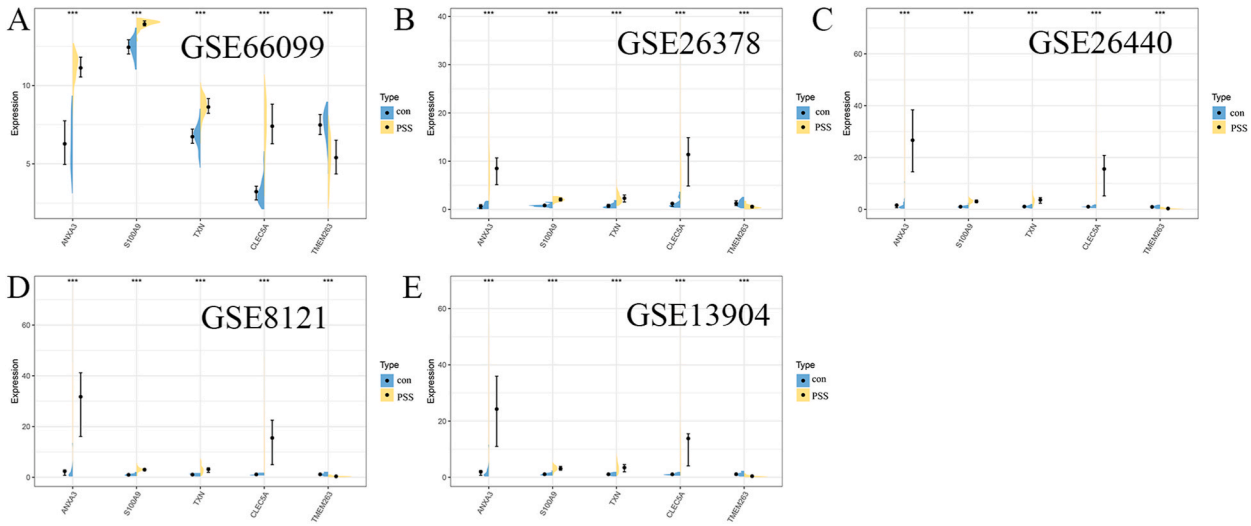

Fig. 8. Five diagnostic PANoptosis gene expression in discovery and validation cohorts. (A) GSE66099. (B) GSE26378. (C) GSE26440. (D) GSE8121. (E) GSE13904. PSS: pediatric septic shock. (***, P < 0.001).

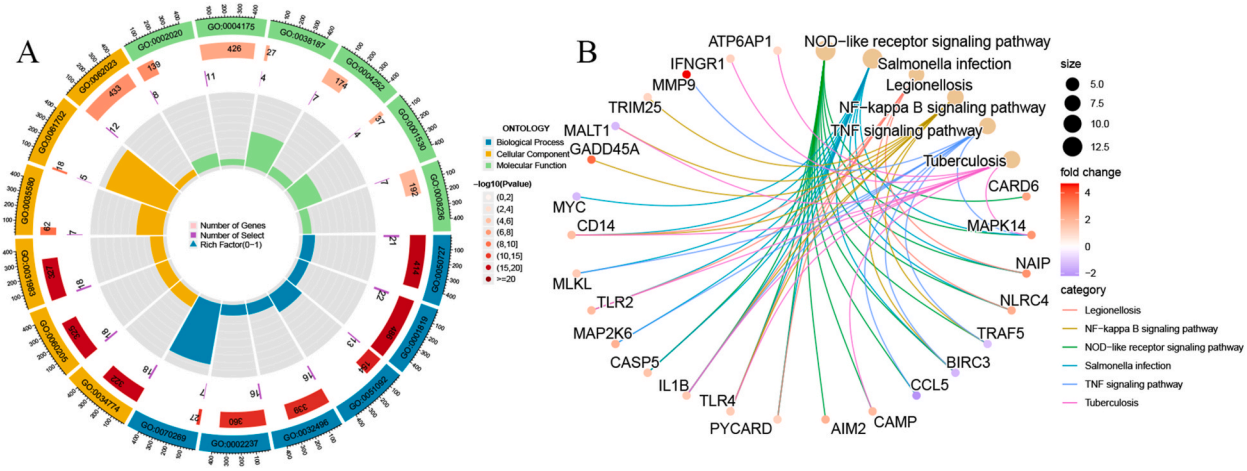

Fig. 9. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes analysis (KEGG) enrichment of 89 differentially expressed PANoptosis genes (DEGs) in GSE66099. (A) Circlize plot showed the top six GO terms and corresponding gene counts of BP, CC, and MF. (B) The relationship between genes and the top six enrichment pathways was represented visually by Cneplot. (P < 0.05)-.

these genes in pediatric septic shock. All DEGs were mostly linked to the regulation of the inflammatory response, the positive regulation of cytokine production, and other related processes, particularly the response to lipopolysaccharides and molecules with bacterial origin, according to a subsequent GO enrichment analysis. Gram-negative bacteria emit endotoxin lipopolysaccharide (LPS) in response to bacterial multiplication, however, this release is significantly enhanced upon bacterial cell death. Numerous factors, including the direct impact of bacterial toxins like endotoxin, might result in septic shock [29]. High blood endotoxin activity is present in between one-third and half of septic shock patients [30]. There was some link found by the KEGG enrichment study between Legionellosis, TNF signaling pathway, NF-kappa B signaling pathway, and Salmonella infection, among other conditions. The findings suggest a connection between these PANoptosis genes and the infection pathways of dangerous pathogenic bacteria.

As for these biomarkers, neutrophils are the only cells that express Annexin A3 (ANXA3), a member of the calcium-binding protein family. Abundance increases of ANXA3 were seen in almost every age group, including neonates. Sepsis, septic shock, and lung damage caused by sepsis are all positively impacted by ANXA3 [31,32]. Thus, we need to revisit and investigate the function of ANXA3 in neutrophils in the context of sepsis and septic shock. Small proteins known as S100 proteins are exclusively expressed in vertebrates. They are involved in the control of calcium homeostasis, glucose metabolism, cell division, apoptosis, inflammation, and carcinogenesis. S100A9 is essential for immunological homeostasis maintenance and microbial infection resistance [33–37]. The TXN gene regulates the differentiation and function of B cells and is associated with heart damage caused by severe inflammation [38,39]. In monocytes, macrophages, neutrophils, and dendritic cells, CLEC5A is extensively expressed and regulates the activation of

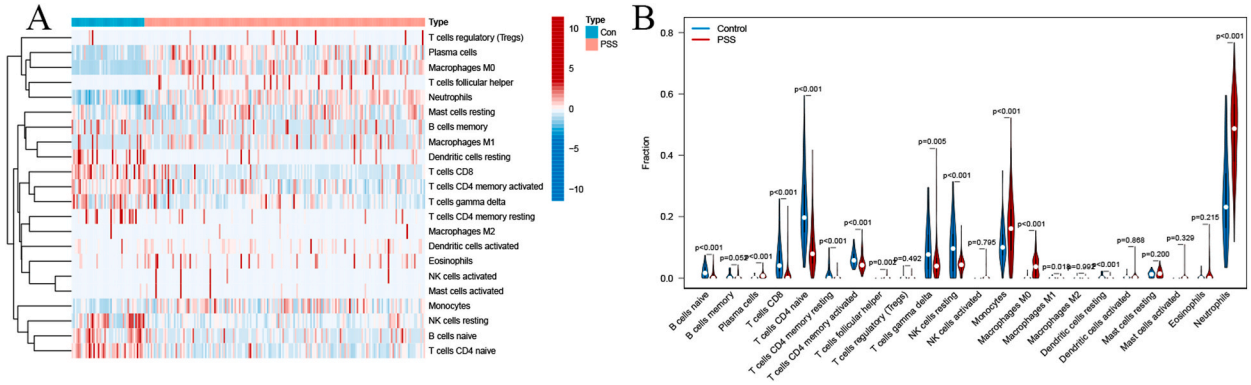

Fig. 10. The 22 subsets of immune cells in pediatric septic shock and healthy control were calculated by CIBERSORT (A) A heatmap displaying each sample's 22 distinct immune cell subgroups. (B) The difference in immune cell infiltration between the cohort of children in septic shock and the healthy group. PSS: pediatric septic shock. ($P < 0.05$).

inflammatory bodies. One possible therapeutic target for infectious disorders is CLEC5A [40–42]. As such, there may be a complex relationship between these marker genes and the development of septic shock in children. To verify these predictions, more investigation is necessary.

In the current study, the immune infiltration process during pediatric sepsis shock was analyzed using the CIBERSORT. This work was motivated by the need to obtain a deeper comprehension of the consequences resulting from immune cell infiltration in pediatric sepsis shock. The occurrence and development of sepsis shock in children may be related to alterations in the infiltration of different immune cells. Based on our investigation, we discovered that pediatric sepsis shock is generally associated with increased levels of neutrophils, monocytes, Macrophages M0, Macrophages M1, T cells follicular helper, and plasma cells. Research has revealed a correlation between the harmful effects of infectious diseases and the quantity of neutrophils in circulation, proinflammatory cytokines generated by macrophages, and monocyte expression [43–46]. Thus, from the standpoint of the immune system, assessing immune cell infiltration and characterizing the variety of immune cell components that are invading are essential for exposing molecular-level causative linkages and developing novel targets for immunotherapy in pediatric septic shock. Thus, more research is desperately needed to assess the function of various immune cells in the immunology generated by septic shock in children.

To summarize, the current investigation of five signature genes: ANXA3, S100A9, TXN, CLEC5A, and TMEM263, demonstrated significant utility in the early detection of pediatric septic shock. A fresh viewpoint on the function of immunity in pediatric septic shock was also supplied by our investigation of the immune cell infiltration in children suffering from septic shock.

There were certain restrictions on our investigation. Even though we used five cohorts to find and validate signature genes, more data are still required. To further support the validity of these five PANoptosis genes, an additional study will be conducted to validate the existing clinical data from our institution externally in the next phase.

5. Conclusion

In summary, our study shows that ANXA3, S100A9, TXN, CLEC5A, and TMEM263 are key DEGs and candidate diagnostic markers in early pediatric septic shock compared to healthy children. ANXA3, TXN, CLEC5A, and TMEM263 were first elucidated in the study of septic shock in children. The study's findings offer information about possible novel molecular biomarkers for pediatric septic shock. The diagnostic value of five biomarkers was developed and validated using five datasets, which increased the reliability of the screening results. In the meantime, we must carry out additional clinical studies in the following phase to validate the current findings.

Ethical approval

Not applicable, all data are publicly available from GEO.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Jing Wang: Writing – original draft, Visualization, Data curation. **ShiFeng Chen:** Investigation, Data curation. **Lei Chen:** Writing – review & editing, Visualization. **Dajie Zhou:** Writing – review & editing, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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