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Research Article
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Development and validation of novel inflammatory response-related gene signature for sepsis prognosis

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Abstract: Due to the low specificity and sensitivity of biomarkers in sepsis diagnostics, the prognosis of sepsis patient outcomes still relies on the assessment of clinical symptoms. Inflammatory response is crucial to sepsis onset and progression; however, the significance of inflammatory response-related genes (IRRGs) in sepsis prognosis is uncertain. This study developed an IRRG-based signature for sepsis prognosis and immunological function. The Gene Expression Omnibus (GEO) database was retrieved for two sepsis microarray datasets, GSE64457 and GSE69528, followed by gene set enrichment analysis (GSEA) comparing sepsis and healthy samples. A predictive signature for IRRGs was created using least absolute shrinkage and selection operator (LASSO). To confirm the efficacy and reliability of the new prognostic signature, Cox regression, Kaplan-Meier (K-M) survival, and receiver operating characteristic (ROC) curve analyses were performed. Subsequently, we employed the GSE95233 dataset to independently validate the prognostic signature. A single-sample GSEA (ssGSEA) was conducted to quantify the immune cell enrichment score and immune-related pathway activity. We found that more gene sets were enriched in the inflammatory response in sepsis patient samples than in healthy patient samples, as determined by GSEA. The signature of nine IRRGs permitted the patients to be classified into two risk categories. Patients in the low-risk group showed significantly better 28-d survival than those in the high-risk group. ROC curve analysis corroborated the predictive capacity of the signature, with the area under the curve (AUC) for 28-d survival reaching 0.866. Meanwhile, the ssGSEA showed that the two risk groups had different immune states. The validation set and external dataset showed that the signature was clinically predictive. In conclusion, a signature consisting of nine IRRGs can be utilized to predict prognosis and influence the immunological status of sepsis patients. Thus, intervention based on these IRRGs may become a therapeutic option in the future.

Key words: Gene signature; Inflammatory response-related gene (IRRG); Prognosis; Immune function; Sepsis

1 Introduction

Sepsis comprises a series of aberrant body reactions induced by infection, resulting in life-threatening organ damage with a high fatality and disability rate (Rhodes et al., 2017). Due to its quick progression and grave prognosis, sepsis has become one of the most prevalent acute and critical diseases among patients undergoing major surgery or suffering burns (Fleischmann-Struzek et al., 2020; Evans et al., 2021). Despite the ongoing advancement of comprehensive treatment methods such as early fluid resuscitation and organ function support therapy, sepsis remains one of the leading causes of death in intensive care units (ICUs) (Gotts and Matthay, 2016). It is predicted to affect at least 48.9 million people annually, with a death rate of up to 30% (Fleischmann et al., 2016; Rhee et al., 2017; Markwart et al., 2020). The Surviving Sepsis Campaign (SSC) has emphasized that the early diagnosis and prompt treatment of sepsis are crucial for improving prognosis (Levy et al., 2018). In light of these facts, there is a significant need in clinical practice for an efficient prognostic biomarker that is able to reliably predict the clinical prognosis of sepsis patients.

The onset and progression of sepsis are complicated processes that frequently involve the pathophysiological alterations of a number of organs and body systems. It is believed that sepsis is a sickness that

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goes through two phases and has a close connection with the inflammatory response. The early phase of hyperinflammation, also called "cytokine storm," is characterized by the excessive production of inflammatory chemicals by the innate immune system, which may or may not result in tissue necrosis (Bosmann and Ward, 2013; Delano and Ward, 2016). Shortly after this exaggerated inflammation response, the immune system dampens, resulting in a state of hypoinflammation. In this condition, both lymphoid and myeloid cells of the immune system become exhausted and go through apoptosis, leaving individuals immunocompromised (van der Poll et al., 2017; Silwal et al., 2020). Multiple studies have sought to integrate pro-inflammatory and anti-inflammatory markers, which is the most likely strategy to succeed in predicting sepsis patients' disease progression (Andaluz-Ojeda et al., 2012; Reinhart et al., 2012). However, these indicators are not reliable as predictive tools for sepsis patients because they lack both specificity and sensitivity.

With the rapid advancement of high-throughput sequencing and gene chip technology, genomics, transcriptomics, and proteomics have been progressively used for illness research (Bauer et al., 2009). Similarly, bioinformatics and machine learning analysis approaches have been widely employed in the analysis of illness gene expression profiles, the identification of specific molecular markers, and the development of clinical prognostic prediction models (Cuocolo et al., 2020; Zhong et al., 2021). In light of these methodologies, certain inflammatory response-related genes, also known as IRRGs, have been utilized to predict the prognosis of metastatic disease (Ju et al., 2021; Xiao et al., 2021). On the other hand, the IRRG signature has not been used to date to predict the consequences of sepsis. As a result, we set out to create and validate an IRRG signature to provide a reliable prognosis prediction for sepsis patients, as well as to describe the immunological status of sepsis patients characterized by a varying prognostic risk.

For the purpose of this investigation, we firstly accessed the Gene Expression Omnibus (GEO) database to obtain the gene expression profile and accompanying clinical data of patients suffering from sepsis. Then, we created a prognostic signature using the IRRGs from the training cohort, and validated the stability and reliability of the model using the validation cohort. Finally, we looked at the disparities in immunological state that existed between the two different risk groups.

2 Materials and methods

2.1 Data collection

The RNA-sequencing (RNA-seq) data were acquired from the GEO database (https://www.ncbi.nlm.nih.gov/geo) (Barrett et al., 2013). Two sepsis microarray datasets, GSE64457 (Demaret et al., 2015) and GSE69528 (Pankla et al., 2009), were acquired from GEO. Another two datasets, GSE65682 (Scicluna et al., 2015) and GSE95233 (Venet et al., 2017), were also retrieved from GEO. GSE64457 contained 23 samples including healthy controls (n=8) and sepsis patients (n=15). GSE69528 contained 138 samples including healthy controls (n=55) and sepsis patients (n=83). After excluding 324 healthy controls and sepsis samples that lacked information on 28-d survival, GSE65682 was chosen for further investigation to screen 478 sepsis patients. GSE95233 was used as an external validation set with 28-d survival information of 51 patients with sepsis. All the above sepsis patients met the diagnostic criteria (Singer et al., 2016).

2.2 Gene set enrichment analysis (GSEA)

GSEA (http://software.broadinstitute.org/gsea) was used to compare molecular pathways and processes between healthy controls and sepsis patients (Subramanian et al., 2005). With an adjusted P value less than 0.05, enriched gene sets were considered statistically significant.

2.3 Identification of IRRGs

The "genes defining inflammatory response" gene sets in the Molecular Signatures Database (MSigDB) (http://www.gsea-msigdb.org) were scoured for the IRRGs, which were then extracted for analysis (Liberzon et al., 2015).

2.4 Development and validation of a prognostic IRRG signature

Patients suffering from sepsis were randomly divided into two groups: training group (n=240) and validation group (n=238). First, univariate Cox regressions were used to investigate the relationships

between IRRGs and 28-d survival in the training set. This was performed to determine whether or not there was a significant relationship between the two variables. Second, prognostic IRRGs were discovered by applying least absolute shrinkage and selection operator (LASSO) Cox regression, which eliminated multicollinearity and decreased the number of prognostic IRRGs (Scicluna et al., 2015). LASSO was then used to exclude any variables from the model that had a regression coefficient equal to zero after the shrinkage phase had been completed. A tuning parameter known as lambda (λ) is responsible for determining the amount of shrinkage; for this parameter, higher values indicate a greater amount of shrinkage. Using 10-fold cross-validation, an ideal was chosen when the partial likelihood deviation was the smallest. Third, we utilized multivariate Cox regression analysis to find predictive IRRGs and generated an IRRG signature for sepsis prognosis by utilizing the Akaike information criterion (AIC) with the lowest value. The following equation was used to obtain the risk score: risk score=β gene (1)×EXP gene (1)+β gene (2)×EXP gene (2)+⋯+β gene (n)×EXP gene (n), with EXP gene reflecting the expression level of the discovered IRRGs adjusted by the Z-score, and β representing the coefficient of that specific IRRG in the prior multivariate Cox regression analysis. Based on their risk score, patients with sepsis in the training and validation sets were classified as low-risk or high-risk. Using the "survival" R package, Kaplan-Meier (K-M) analysis was employed to compare 28-d survival between the low- and high-risk groups (Rich et al., 2010). The "pROC" R package was utilized to evaluate the reliability of the signature (Robin et al., 2011).

2.5 Comparison of immune status of sepsis patients from different risk groups

Using single-sample GSEA (ssGSEA), we compared the enrichment scores of immune cells and the activity of immune-related pathways across the low- and high-risk groups. The "gsva" R package (Hänzelmann et al., 2013) was used for this task, and P<0.05 was considered statistically significant.

2.6 Statistical analysis

In order to distinguish IRRGs linked with prognosis, LASSO-Cox regression analysis was utilized. K-M analysis and the log-rank test were used to compare survival between the low- and high-risk groups. The Mann-Whitney test was used to compare ssGSEA scores of immune cells or pathways between the low- and high-risk groups, while adjusting the P values with the Benjamini-Hochberg (B-H) procedure. The predictive accuracy of the prognostic prediction signature was assessed by receiver operating characteristic (ROC) curve analysis. All of these tasks necessitated using version 4.1 of the R programming language. A double-tailed P value less than 0.05 was considered statistically significant.

3 Results

Fig. 1 depicted the flowchart of the investigation. The relevant clinical data of the modeling dataset were presented in Table S1.

3.1 GSEA

The inflammatory response (normalized enrichment score (NES)=1.928, adjusted P<0.05) and regulation of inflammatory response (NES=1.822, adjusted P<0.05) were considerably enriched in sepsis patients (Fig. 2).

3.2 Identification of prognostic IRRGs

The MSigDB had 200 IRRGs, which were listed in Table S2. First, we identified 44 IRRGs that were associated with 28-d survival and had prognostic significance (P<0.05; Fig. 3, Table S3). Second, LASSO-Cox regression analysis was employed to reduce multicollinearity and limit the number of IRRGs to 19 (P<0.05; Figs. 4a and 4b, Table S4). Fig. 4c is a visual representation of the correlation between these IRRGs.

3.3 Development of prognostic IRRG signature

The clinical prognosis prediction model of sepsis was created using nine IRRGs (*CCL22*, *CX3CL1*, *CXCR6*, *FFAR2*, *FPR1*, *HBEGF*, *ITGA5*, *RGS16*, and *SELL*) by multivariate Cox regression analysis and the principle of minimum AIC (Fig. 5, Table 1). The following risk scoring formula was obtained through gene expression and regression coefficient in the model: risk score=1.059×*CCL22*+1.227×*CX3CL1*+0.460×*CXCR6*−0.789×*FFAR2*+2.743×*FPR1*+0.411×

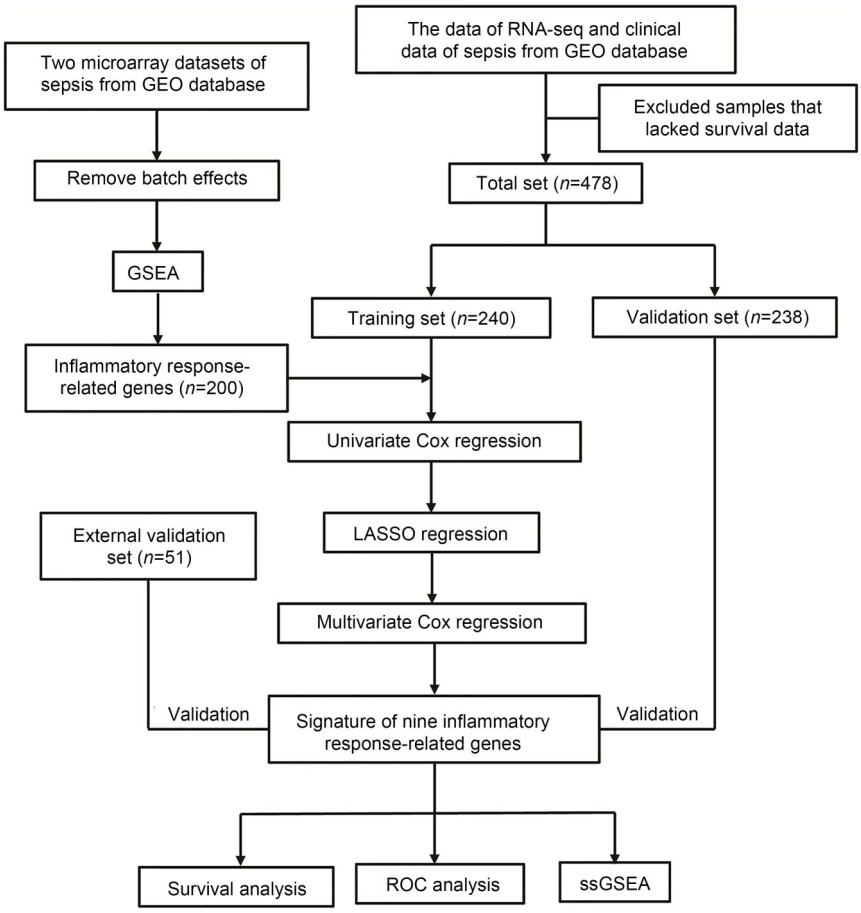

Fig. 1 Flowchart of this study. RNA-seq: RNA-sequencing; GEO: Gene Expression Omnibus; GSEA: gene set enrichment analysis; LASSO: least absolute shrinkage and selection operator; ROC: receiver operating characteristic; ssGSEA: single-sample GSEA.

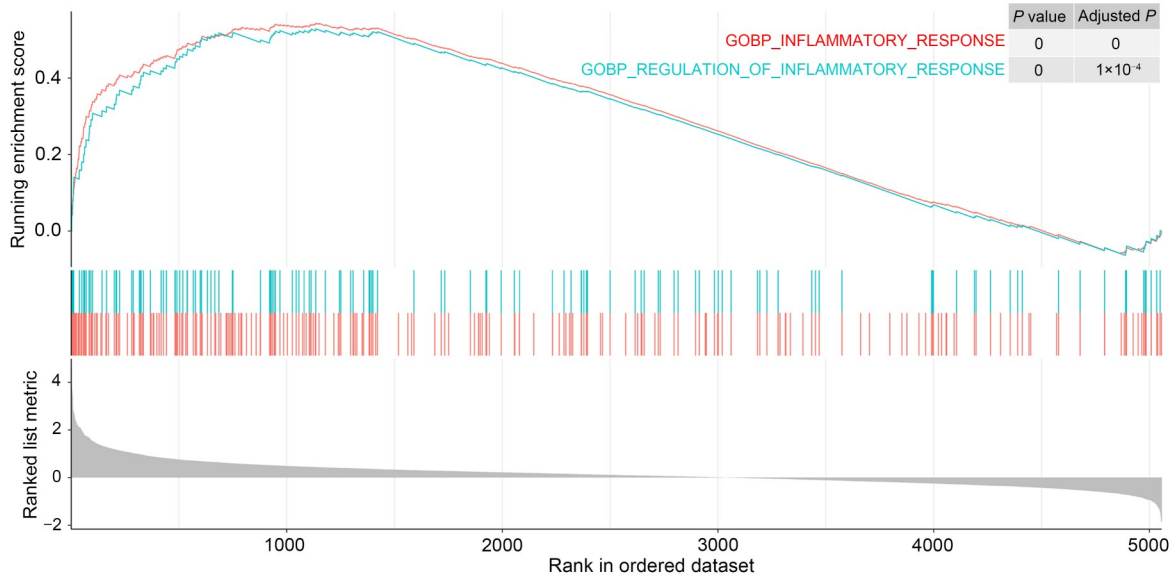

Fig. 2 Enrichment plots from GSEA between normal controls and sepsis samples. GSEA: gene set enrichment analysis; GOBP: gene ontology biological process.

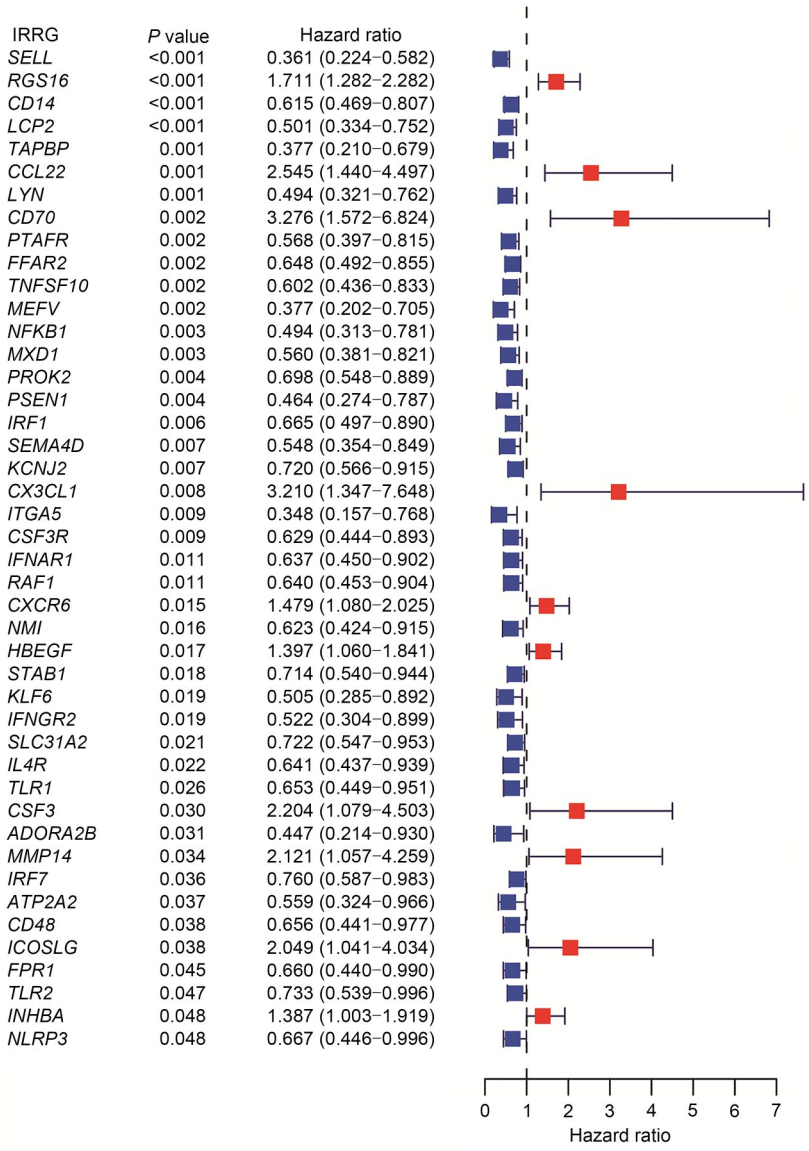

Fig. 3 Identification of the candidate 44 IRRGs associated with 28-d survival in sepsis patients. IGGRs: inflammatory response-related genes.

HBEGF−0.915×*ITGA5*+0.565×*RGS16*−1.733×*SELL* (AIC=581.29, consistency index=0.73; Table S5). Patients in the training set were separated into low-risk (n=120) and high-risk (n=120) groups based on the median patient risk score critical value (Figs. 6a and 6b). The heatmap depicted the nine IRRG expression profiles of each sepsis patient (Fig. 6c). The K-M curve analysis revealed that the low-risk group had considerably higher 28-d survival than the high-risk group (P<0.05; Fig. 6d). The area under curve (AUC) of the ROC of the risk score signature to predict 28-d survival in sepsis patients was 0.866 (P<0.05; Fig. 6e).

3.4 Validation of prognostic signature of IRRGs

Patients in the validation set were divided into low-risk (n=117) and high-risk (n=121) groups based on the same risk score as in the training set (Figs. 7a and 7b). The heatmap showed nine IRRG expression profiles of each patient with sepsis in the validation set (Fig. 7c). K-M curve analysis also indicated that the 28-d survival of the low-risk group was significantly higher than that of the high-risk group in the validation set (P<0.05; Fig. 7d). The AUC of signature to predict 28-d survival in sepsis patients by risk score was 0.858 in the validation set (P<0.05; Fig. 7e).

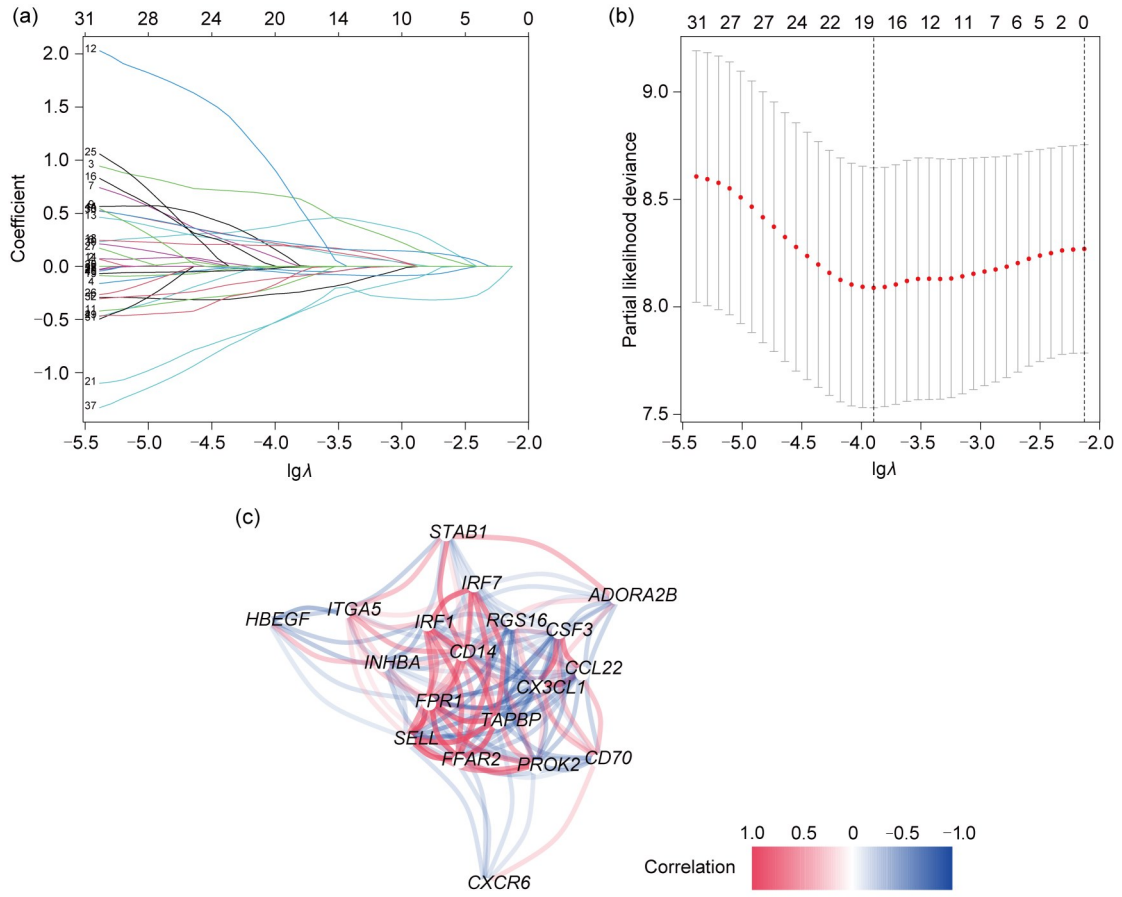

Fig. 4 Construction of an IGGR prognostic signature by LASSO regression analyses. (a) The penalty parameter (λ) of the LASSO model was chosen using ten cross-validation runs. (b) The expression profiles of 19 potential IRRGs using LASSO coefficients. (c) The correlation network of candidate IRRGs. IGGRs: inflammatory response-related genes; LASSO: least absolute shrinkage and selection operator.

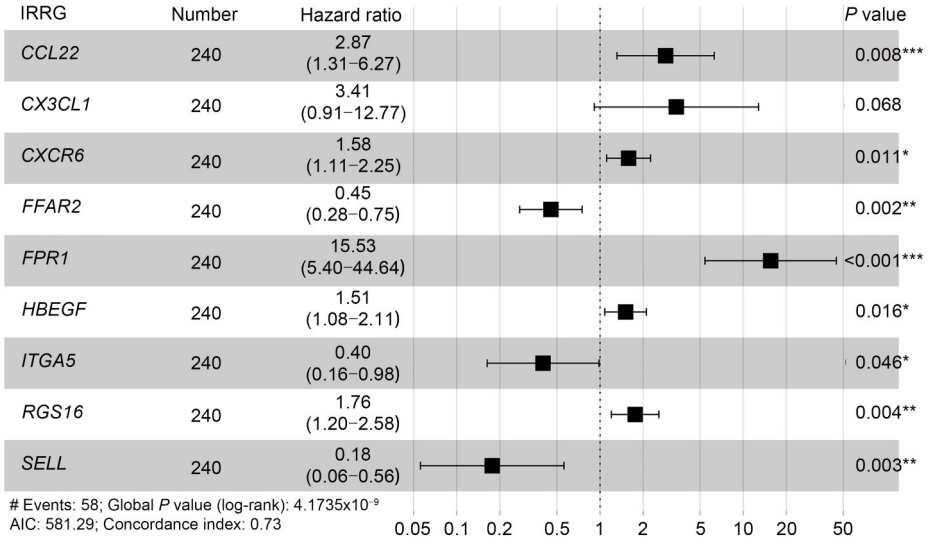

Fig. 5 Forest plot results of the connection between nine IRRGs and 28-d survival in sepsis patients by multivariate Cox regression. * *P*<0.05, ** *P*<0.01, * *P*<0.001. IRRGs: inflammatory response-related genes; AIC: Akaike information criterion.**

Table 1 Construction of prognostic signatures in patients with sepsis

Signature	Combination of prognostic signature	Value of Akaike information criterion
1	*CXCR6+ADORA2B+INHBA+PROK2+CSF3+TAPBP+STAB1+CD70+IRF1+CD14+IRF7*	607.33
2	*CXCR6+FPR1+ADORA2B+INHBA+PROK2+CSF3+TAPBP+STAB1+CD70+IRF1+CD14+IRF7*	588.36
3	*CXCR6+FFAR2+FPR1+ADORA2B+INHBA+PROK2+CSF3+TAPBP+STAB1+CD70+IRF1+CD14+IRF7*	587.88
4	*CXCR6+FFAR2+FPR1+SELL+ADORA2B+INHBA+PROK2+CSF3+TAPBP+STAB1+CD70+IRF1+CD14+IRF7*	585.93
5	*CXCR6+FFAR2+FPR1+RGS16+SELL+ADORA2B+INHBA+PROK2+CSF3+TAPBP+STAB1+CD70+IRF1+CD14+IRF7*	585.90
6	*CCL22+FFAR2+FPR1+RGS16+SELL+ADORA2B+INHBA+PROK2+CSF3+TAPBP+STAB1+CD70+IRF1+CD14+IRF7*	584.54
7	*CCL22+CXCR6+FFAR2+FPR1+RGS16+SELL+ADORA2B+INHBA+PROK2+CSF3+TAPBP+STAB1+CD70+IRF1+CD14+IRF7*	584.33
8	*CCL22+CXCR6+FFAR2+FPR1+HBEGF+RGS16+SELL+ADORA2B+INHBA+PROK2+CSF3+TAPBP+STAB1+CD70+IRF1+CD14+IRF7*	583.61
9	*CCL22+CXCR6+FFAR2+FPR1+HBEGF+ITGA5+RGS16+SELL+ADORA2B+INHBA+PROK2+CSF3+TAPBP+STAB1+CD70+IRF1+CD14+IRF7*	583.29
10	*CCL22+CXCR6+FFAR2+FPR1+HBEGF+ITGA5+RGS16+SELL+ADORA2B+INHBA+PROK2+CSF3+TAPBP+STAB1+CD70+IRF1+CD14*	583.26
11	*CCL22+CXCR6+FFAR2+FPR1+HBEGF+ITGA5+RGS16+SELL+ADORA2B+INHBA+PROK2+CSF3+TAPBP+STAB1+CD70+IRF1*	583.16
12	*CCL22+CXCR6+FFAR2+FPR1+HBEGF+ITGA5+RGS16+SELL+ADORA2B+INHBA+PROK2+CSF3+TAPBP+STAB1+CD70*	583.14
13	*CCL22+CXCR6+FFAR2+FPR1+HBEGF+ITGA5+RGS16+SELL+ADORA2B+INHBA+PROK2+CSF3+TAPBP+STAB1*	582.54
14	*CCL22+CX3CL1+CXCR6+FFAR2+FPR1+HBEGF+ITGA5+RGS16+SELL+ADORA2B+INHBA+PROK2+CSF3+TAPBP+STAB1*	582.36
15	*CCL22+CX3CL1+CXCR6+FFAR2+FPR1+HBEGF+ITGA5+RGS16+SELL+ADORA2B+INHBA+PROK2+CSF3+TAPBP*	582.32
16	*CCL22+CX3CL1+CXCR6+FFAR2+FPR1+HBEGF+ITGA5+RGS16+SELL+ADORA2B+INHBA+PROK2+CSF3*	582.24
17	*CCL22+CX3CL1+CXCR6*+FFAR2+*FPR1+HBEGF+ITGA5+RGS16+SELL+ADORA2B+INHBA+PROK2*	581.82
18	*CCL22+CX3CL1+CXCR6+FFAR2+FPR1+HBEGF+ITGA5+RGS16+SELL+ADORA2B+INHBA*	581.60
19	*CCL22+CX3CL1+CXCR6+FFAR2+FPR1+HBEGF+ITGA5+RGS16+SELL+ADORA2B*	581.48
20	*CCL22+CX3CL1+CXCR6+FFAR2+FPR1+HBEGF+ITGA5+RGS16+SELL*	581.29

The verification of the external dataset showed that the 28-d survival of the low-risk group was significantly higher than that of the high-risk group (P<0.05; Fig. 8a), and the AUC of signature to predict 28-d survival in sepsis patients by risk score was 0.813 (P< 0.05; Fig. 8b).

3.5 Comparison of immune status of sepsis patients from different risk groups

We next investigated the immune cell enrichment scores using the ssGSEA results. Patients with sepsis in the high-risk group had higher enrichment scores of dendritic cells (DCs) and immature DCs (iDCs) than those in the low-risk group, while they had lower enrichment scores of neutrophils, natural killer (NK) cells, plasmacytoid DCs (pDCs), and tumor infiltrating lymphocytes (TILs) than those in the low-risk group (P<0.05; Fig. 9a). As for immune functions, patients with sepsis in the high-risk group had higher enrichment scores of type II inborn errors of interferon (IFN) response than those in the low-risk group, and they had lower enrichment scores of C-C motif chemokine receptor (CCR), checkpoint, human leukocyte antigen (HLA), inflammation-promoting, major histocompatibility complex (MHC) class I, parainflammation, T cell co-inhibition, T cell co-stimulation, and Type I IFN response than those in the low-risk group (P<0.05; Fig. 9b). Fig. 9c presents a comparison of the immunological checkpoints of sepsis patients that fell into one of several different risk categories.

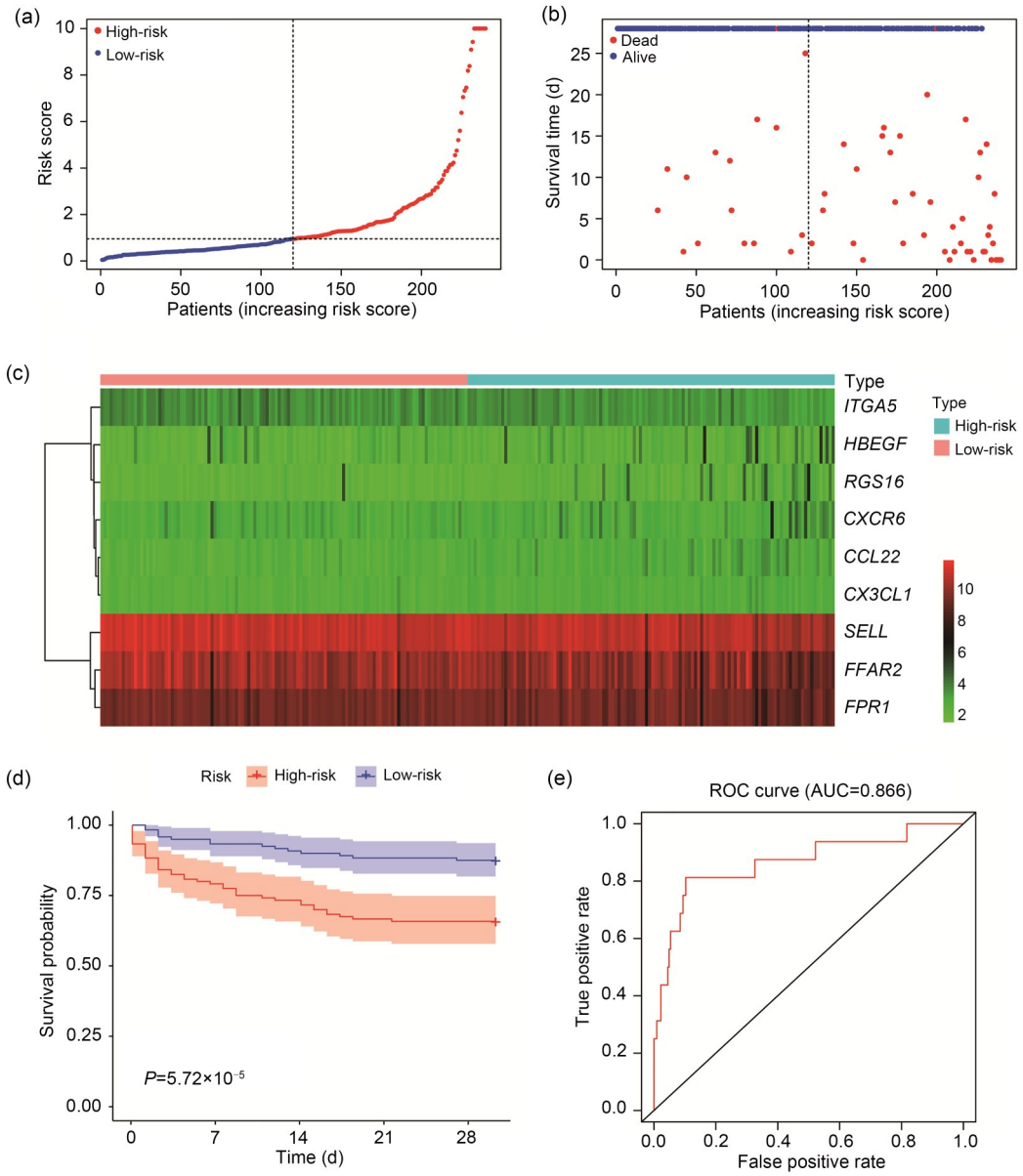

Fig. 6 Prognostic performance of nine IRRG signatures in the training set. (a) The median value and distribution of the risk scores calculated by this signature. (b) The distribution of 28-d survival status. (c) The expression of nine IRRGs between the low- and high-risk groups. (d) Kaplan-Meier curves of the 28-d survival based on the risk score of sepsis patients. (e) The prognostic accuracy of the risk score confirmed by the AUC of ROC. IRRGs: inflammatory response-related genes; AUC: area under the curve; ROC: receiver operating characteristic.

4 Discussion

Sepsis involves the body's dysregulated immune reaction to infection, which causes life-threatening organ dysfunction. The situation rapidly deteriorates and may develop to septic shock and multiple organ failure, resulting in persistent tissue hypoperfusion, cell malfunction, and abnormal body metabolism, all of which represent a significant death risk (Winters et al., 2010; Delano and Ward, 2016; Rhee et al., 2017). Because there are few reliable markers, it is not always possible to detect and predict the consequences of sepsis treatment. The genetic signatures of numerous solid tumors have been correlated with a variety of clinical outcomes to improve clinical care through the creation of personalized medicine (Zou

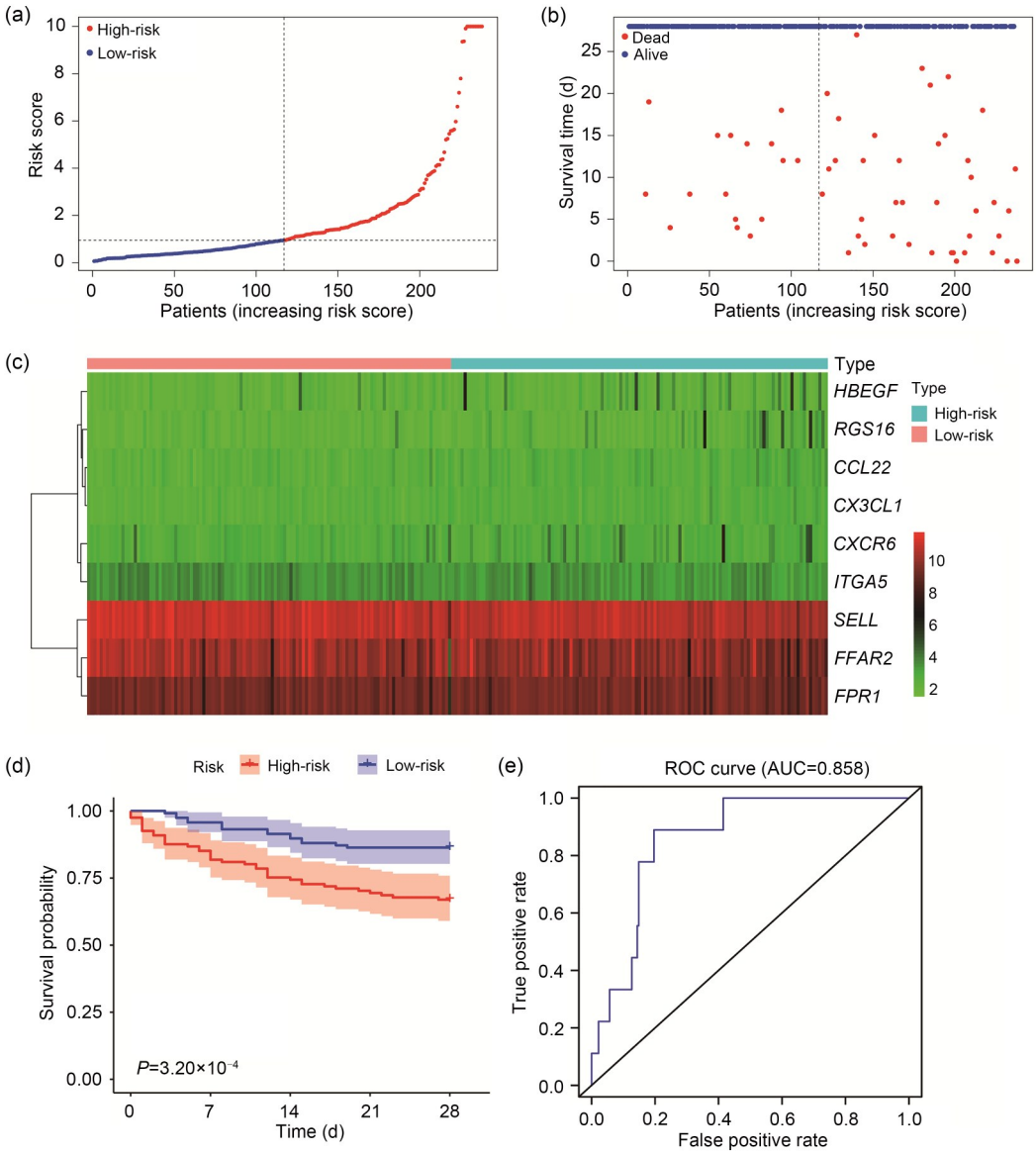

Fig. 7 Prognostic performance of nine IRRG signatures in the validation set. (a) The median value and distribution of the risk scores calculated by this signature. (b) The distribution of 28-d survival status. (c) The expression of nine IRRGs between the low- and high-risk groups. (d) Kaplan-Meier curves of the 28-d survival based on the risk score of sepsis patients. (e) The prognostic accuracy of the risk score confirmed by the AUC of ROC. IRRGs: inflammatory response-related genes; AUC: area under the curve; ROC: receiver operating characteristic.

et al., 2021; Deng et al., 2022). As a result, it is imperative to carry out extensive research on impact biomarkers to improve the clinical care and prognosis of sepsis patients.

Serum indicators associated with the inflammatory response, such as the neutrophil-lymphocyte ratio, platelet-lymphocyte ratio, lymphocyte-monocyte ratio, procalcitonin, and C-reactive protein, have been linked to the prognosis of sepsis (Ruan et al., 2018; Kriplani et al., 2022). In terms of detail and sensitivity, however, their performances fall short. Unfortunately, no research has been performed on the IRRG signature as a predictor of sepsis. Numerous studies have showed that *S1PR3* gene signatures closely predict the prognosis of sepsis (Feng et al., 2021). In addition to its superior effectiveness in sepsis prognosis, the IRRG signature developed in this study provides other advantages over the previously described gene signature. For instance, it may define IRRGs as low- or high-expression, and establishes correlations between

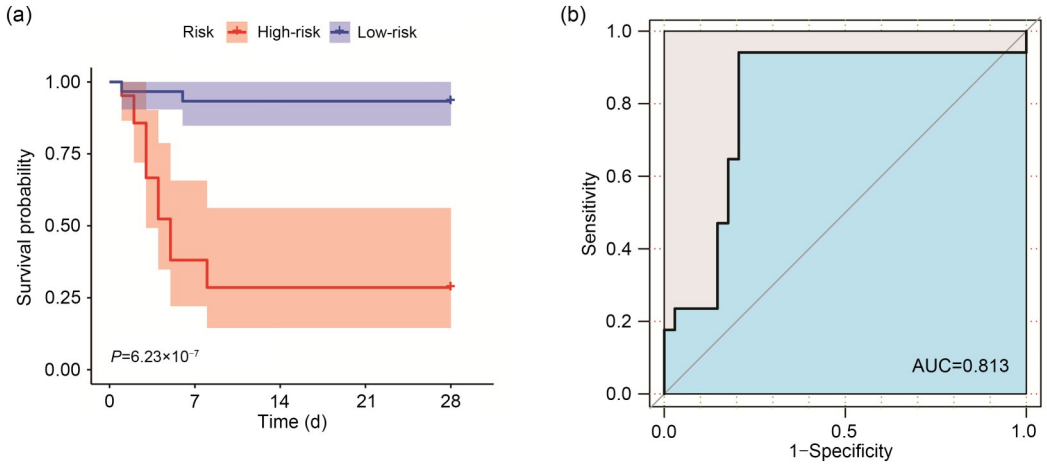

Fig. 8 Prognostic performance of nine IRRG signatures in the external validation set. (a) Kaplan-Meier curves depicting the 28-d survival based on the risk score of sepsis patients. (b) The prognostic accuracy of the risk score was confirmed by the AUC of ROC. IRRGs: inflammatory response-related genes; AUC: area under the curve; ROC: receiver operating characteristic.

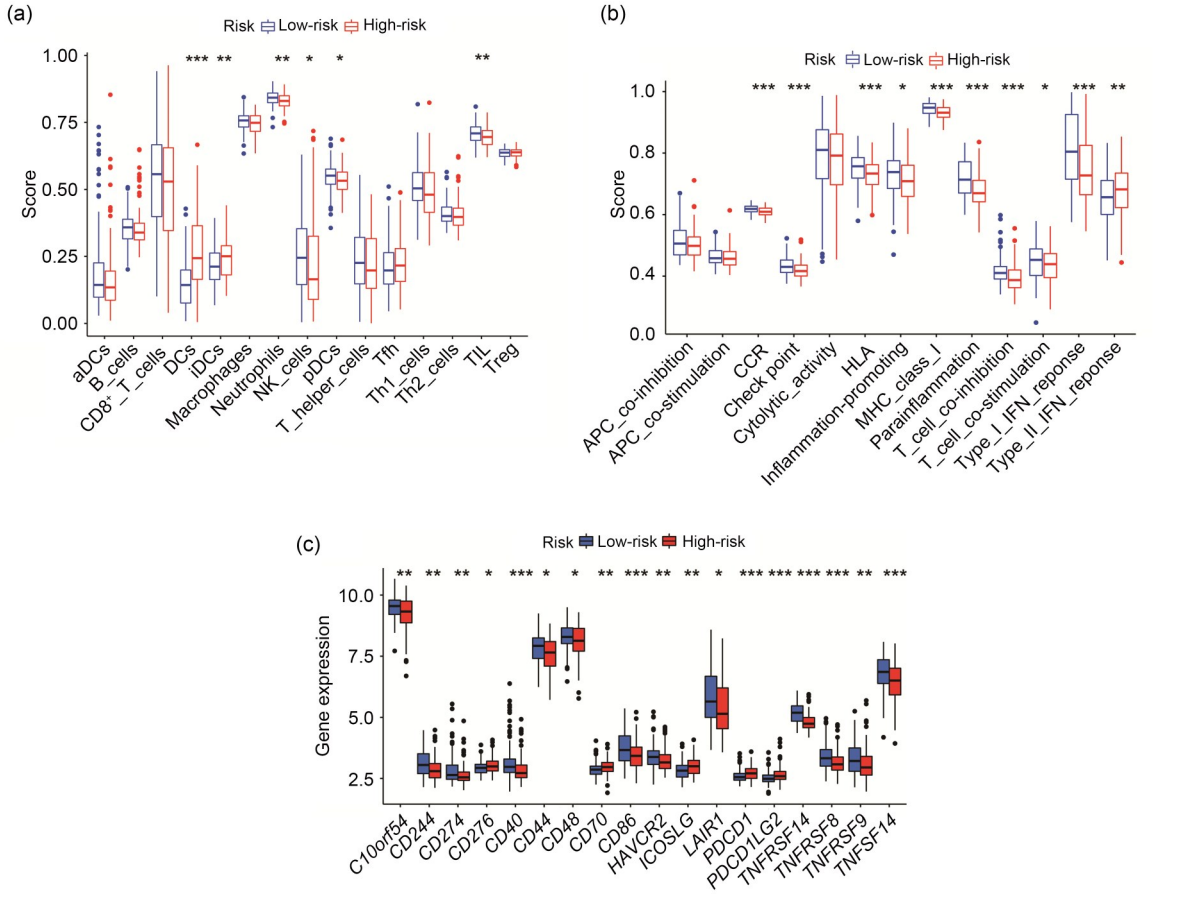

Fig. 9 Comparison of ssGSEA scores of sepsis patients in different risk groups. (a) The scores of immune cells. (b) The scores of immune functions. (c) The expression levels of immune checkpoints. $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001. ssGSEA: single-sample gene set enrichment analysis; DCs: dendritic cells; aDCs: actived DCs; iDCs: immature DCs; pDCs: plasmacytoid DCs; NK: natural killer; Tfh: follicular helper T cell; Th: help T cell; TIL: tumor infiltrating lymphocyte; Treg: regularoty T cell; APC: antigen presenting cell; CCR: C-C motif chemokine receptor; HLA: human leukocyte antigen; MHC: major histocompatibility complex; IFN: interferon.

risk ratings and 28-d survival. In this study, the expression levels of prognostic signature genes were determined using high-throughput sequencing, which is a commonly utilized technology that yields accurate results.

In this work, we evaluated the expression of 200 genes associated with the inflammatory response and 28-d survival in sepsis patients. The univariate Cox analysis of the training cohort revealed that 44 IRRGs were linked with 28-d survival. Using LASSO and multivariate Cox regression approaches, a prognostic signature was established. The nine IRRGs in the signature were validated against the validation set and external dataset. Based on the median risk score, participants were categorized as low- or high-risk groups. The results suggested that the high-risk group had a lower 28-d survival rate. In both the training and validation sets, a distinct IRRG prognostic signature revealed small predictive value for the survival of sepsis patients based on ROC analysis.

All of these nine IRRG signatures that might predict the prognosis of sepsis patients, *CCL22*, *CX3CL1*, *CXCR6*, *FFAR2*, *FPR1*, *HBEGF*, *ITGA5*, *RGS16*, and *SELL* had been studied extensively in prior research. It is usually considered that *CCL22*, a kind of anti-inflammatory (M2) chemokine, plays a role in the transition of monocytes and macrophages to the M2 phenotype, which may contribute to sepsis-induced immunosuppression (Watanabe et al., 2016). However, no study has established a link between *CCL22* and sepsis prognosis. *CX3CL1*, also known as fractalkine, is essential for chemokine cell directionality, immune response, inflammatory response, and tissue healing among other tasks (Helmke et al., 2019; Korbecki et al., 2020; Ni et al., 2020). Basic experiments showed that the *CX3CL1*/*CX3CR1* axis may reduce the release of proinflammatory cytokines in sepsis mice through the downregulation of *CX3CR1* mediated by transcription factor nuclear factor-κB (NF-κB) (Raspé et al., 2013). According to one study, blocking *FFAR2* may play an important role in sepsis and contribute to the pathophysiology of the inflammatory response it causes (Schlatterer et al., 2021a). The induction of chemotaxis towards local areas of infection or inflammation is the most well-described and established effector function of *FFAR2* activation in neutrophils, although the effect on other neutrophil functions, such as the release of cytokines, is less evident. In addition, contradictory evidence has been found on *FFAR2*-dependent cytokine and antimicrobial peptide production with either stimulatory or inhibitory effects (Schlatterer et al., 2021b). *FPR1* may limit neutrophil antimicrobial action, hence alleviating the systemic inflammatory response syndrome and defending the body against subsequent infection following tissue trauma or initial infection (Itagaki et al., 2020). A previous study has suggested that *RGS16* overexpression may also be linked to the early onset of cardiac failure in sepsis (Patten et al., 2002). *CXCR6* may serve as a marker for malignancies that emerge in an inflammatory environment, and mediate the protumorigenic effects of inflammation through direct effects on cancer cell growth and by stimulating the migration and proliferation of tumor-associated leukocytes (Darash-Yahana et al., 2009). *HBEGF* has the potential to activate NF-κB, which would result in increased levels of interleukin-6 (IL-6) and IL-8 expression (Miyata et al., 2012). *ITGA5* is a microRNA-92a (miR-92a) target gene, and miR-92a suppression alleviates the inflammatory response by reducing the release of proinflammatory cytokines IL-6 and tumor necrosis factor-α (TNF-α) (Xu and Zhou, 2020). L-Selectin, as a cell surface adhesion molecule and a member of the adhesion/homing receptor family, promotes leukocyte migration to lymphoid organs, which is related to the severity of inflammation, and is encoded by the *SELL* gene (Wedepohl et al., 2012). In sepsis, the underlying mechanism of *CXCR6*, *HBEGF*, *ITGA5*, or *SELL* IRRG has not been fully elucidated.

The presence of inflammation results in an immunosuppressive environment, which speeds up the course of sepsis (Hotchkiss et al., 2013; Venet and Monneret, 2018). We revealed that patients in the high-risk group had higher enrichment scores of DCs and iDCs than those in the low-risk group, and they had lower enrichment scores of neutrophils, NK cells, pDCs, and TIL than those in the low-risk group. Bouras et al. (2018) found that the presence of DCs in the blood was linked to increased chances of survival in sepsis patients. Neutrophils, which are the most common type of leukocytes found in the bloodstream, play roles in both inflammatory and immune responses (Kolaczkowska and Kubes, 2013). Hanna et al. (2019) found that decreased neutrophil CD16 expression was associated with a higher risk of death in severely ill sepsis patients. According to previous research, morphological

and functional changes in neutrophils during sepsis decreased bacterial clearance and predispose sepsis patients to nosocomial infections, resulting in poor prognosis (He et al., 2021; Lu et al., 2022). NK cells are also a subtype of lymphocytes known as innate lymphoid cells, which are essential for initiating host defenses and coordinating innate and adaptive immune responses. During sepsis, NK cells undergo quantitative, morphological, and functional alterations. A considerable decline in the number of circulating NK cells is associated with an increased risk of death in sepsis patients, probably due to increased apoptosis (Jensen et al., 2018).

Our investigation has some constraints, inevitably. First, we began by evaluating the clinical efficacy of our prognostic signature without using additional prospective real-world data. Second, due to a paucity of relevant clinical data, we were unable to incorporate all critical clinical data into our predictive model. Finally, it was acknowledged that associations between the risk score and immunological condition must be empirically validated.

5 Conclusions

Our research established a novel predictive signature of nine IRRGs for the prognosis of sepsis. This signature was found to be independently associated with 28-d survival, providing a valuable tool for the prediction of sepsis prognosis. Immune status variations were observed to be associated with the progression of sepsis. Thus, the proposed signature may be developed into a useful biomarker in the future for the reliable prediction of sepsis prognosis.

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Author contributions

Shuai JIANG and Wenyuan ZHANG conceived the idea and performed data analyses, wrote and edited the manuscript. Yuanqiang LU contributed to the study design, data analysis, and writing and editing of the manuscript. All authors have read and approved the final manuscript, and therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Shuai JIANG, Wenyuan ZHANG, and Yuanqiang LU declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Andaluz-Ojeda D, Bobillo F, Iglesias V, et al., 2012. A combined score of pro- and anti-inflammatory interleukins improves mortality prediction in severe sepsis. *Cytokine*, 57(3):332-336. https://doi.org/10.1016/j.cyto.2011.12.002

Barrett T, Wilhite SE, Ledoux P, et al., 2013. NCBI GEO: archive for functional genomics data sets—update. *Nucleic Acids Res*, 41(D1):D991-D995. https://doi.org/10.1093/nar/gks1193

Bauer JW, Bilgic H, Baechler EC, 2009. Gene-expression profiling in rheumatic disease: tools and therapeutic potential. *Nat Rev Rheumatol*, 5(5):257-265. https://doi.org/10.1038/nrrheum.2009.50

Bosmann M, Ward PA, 2013. The inflammatory response in sepsis. *Trends Immunol*, 34(3):129-136. https://doi.org/10.1016/j.it.2012.09.004

Bouras M, Asehnoune K, Roquilly A, 2018. Contribution of dendritic cell responses to sepsis-induced immunosuppression and to susceptibility to secondary pneumonia. *Front Immunol*, 9:2590. https://doi.org/10.3389/fimmu.2018.02590

Cuocolo R, Caruso M, Perillo T, et al., 2020. Machine learning in oncology: a clinical appraisal. *Cancer Lett*, 481:55-62. https://doi.org/10.1016/j.canlet.2020.03.032

Darash-Yahana M, Gillespie JW, Hewitt SM, et al., 2009. The chemokine CXCL16 and its receptor, CXCR6, as markers and promoters of inflammation-associated cancers. *PLoS ONE*, 4(8):e6695. https://doi.org/10.1371/journal.pone.0006695

Delano MJ, Ward PA, 2016. The immune system's role in sepsis progression, resolution, and long-term outcome. *Immunol Rev*, 274(1):330-353. https://doi.org/10.1111/imr.12499

Demaret J, Venet F, Friggeri A, et al., 2015. Marked alterations of neutrophil functions during sepsis-induced immunosuppression. *J Leukoc Biol*, 98(6):1081-1090. https://doi.org/10.1189/jlb.4A0415-168RR

Deng ZL, Zhou DZ, Cao SJ, et al., 2022. Development and validation of an inflammatory response-related gene signature for predicting the prognosis of pancreatic adenocarcinoma. *Inflammation*, 45(4):1732-1751. https://doi.org/10.1007/s10753-022-01657-6

Evans L, Rhodes A, Alhazzani W, et al., 2021. Executive summary: Surviving Sepsis Campaign: International Guidelines for the Management of Sepsis and Septic Shock 2021. *Crit Care Med*, 49(11):1974-1982. https://doi.org/10.1097/CCM.0000000000005357

Feng AL, Ma WL, Faraj R, et al., 2021. Identification of S1PR3 gene signature involved in survival of sepsis patients. *BMC Med Genomics*, 14:43.

https://doi.org/10.1186/s12920-021-00886-2

Fleischmann C, Scherag A, Adhikari NKJ, et al., 2016. Assessment of global incidence and mortality of hospital-treated sepsis. Current estimates and limitations. *Am J Respir Crit Care Med*, 193(3):259-272. https://doi.org/10.1164/rccm.201504-0781OC

Fleischmann-Struzek C, Mellhammar L, Rose N, et al., 2020. Incidence and mortality of hospital- and ICU-treated sepsis: results from an updated and expanded systematic review and meta-analysis. *Intensive Care Med*, 46(8):1552-1562. https://doi.org/10.1007/s00134-020-06151-x

Gotts JE, Matthay MA, 2016. Sepsis: pathophysiology and clinical management. *BMJ*, 353:i1585. https://doi.org/10.1136/bmj.i1585

Hanna MOF, Abdelhameed AM, Abou-Elalla AA, et al., 2019. Neutrophil and monocyte receptor expression in patients with sepsis: implications for diagnosis and prognosis of sepsis. *Pathog Dis*, 77(6):ftz055. https://doi.org/10.1093/femspd/ftz055

Hänzelmann S, Castelo R, Guinney J, 2013. GSVA: gene set variation analysis for microarray and RNA-Seq data. *BMC Bioinformatics*, 14:7. https://doi.org/10.1186/1471-2105-14-7

He WX, Xiao K, Fang M, et al., 2021. Immune cell number, phenotype, and function in the elderly with sepsis. *Aging Dis*, 12(1):277-296. https://doi.org/10.14336/Ad.2020.0627

Helmke A, Nordlohne J, Balzer MS, et al., 2019. CX3CL1–CX3CR1 interaction mediates macrophage-mesothelial cross talk and promotes peritoneal fibrosis. *Kidney Int*, 95(6):1405-1417. https://doi.org/10.1016/j.kint.2018.12.030

Hotchkiss RS, Monneret G, Payen D, 2013. Sepsis-induced immunosuppression: from cellular dysfunctions to immunotherapy. *Nat Rev Immunol*, 13(12):862-874. https://doi.org/10.1038/nri3552

Itagaki K, Kaczmarek E, Kwon WY, et al., 2020. Formyl peptide receptor-1 blockade prevents receptor regulation by mitochondrial danger-associated molecular patterns and preserves neutrophil function after trauma. *Crit Care Med*, 48(2):e123-e132. https://doi.org/10.1097/Ccm.0000000000004094

Jensen IJ, Winborn CS, Fosdick MG, et al., 2018. Polymicrobial sepsis influences NK-cell-mediated immunity by diminishing NK-cell-intrinsic receptor-mediated effector responses to viral ligands or infections. *PLoS Pathog*, 14(10):e1007405. https://doi.org/10.1371/journal.ppat.1007405

Ju AJ, Tang JZ, Chen SH, et al., 2021. Pyroptosis-related gene signatures can robustly diagnose skin cutaneous melanoma and predict the prognosis. *Front Oncol*, 11:709077. https://doi.org/10.3389/fonc.2021.709077

Kolaczkowska E, Kubes P, 2013. Neutrophil recruitment and function in health and inflammation. *Nat Rev Immunol*, 13(3):159-175. https://doi.org/10.1038/nri3399

Korbecki J, Simińska D, Kojder K, et al., 2020. Fractalkine/CX3CL1 in neoplastic processes. *Int J Mol Sci*, 21(10):3723. https://doi.org/10.3390/ijms21103723

Kriplani A, Pandit S, Chawla A, et al., 2022. Neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR) and lymphocyte-monocyte ratio (LMR) in predicting systemic inflammatory response syndrome (SIRS) and sepsis after percutaneous nephrolithotomy (PNL). *Urolithiasis*, 50(3):341-348. https://doi.org/10.1007/s00240-022-01319-0

Levy MM, Evans LE, Rhodes A, 2018. The surviving sepsis campaign bundle: 2018 update. *Crit Care Med*, 46(6):997-1000. https://doi.org/10.1097/Ccm.0000000000003119

Liberzon A, Birger C, Thorvaldsdóttir H, et al., 2015. The molecular signatures database (MSigDB) hallmark gene set collection. *Cell Syst*, 1(6):417-425. https://doi.org/10.1016/j.cels.2015.12.004

Lu JQ, Liu JY, Li A, 2022. Roles of neutrophil reactive oxygen species (ROS) generation in organ function impairment in sepsis. *J Zhejiang Univ-Sci B (Biomed & Biotechnol)*, 23(6):437-450. https://doi.org/10.1631/jzus.B2101075

Markwart R, Saito H, Harder T, et al., 2020. Epidemiology and burden of sepsis acquired in hospitals and intensive care units: a systematic review and meta-analysis. *Intensive Care Med*, 46(8):1536-1551. https://doi.org/10.1007/s00134-020-06106-2

Miyata K, Yotsumoto F, Nam SO, et al., 2012. Regulatory mechanisms of the HB-EGF autocrine loop in inflammation, homeostasis, development and cancer. *Anticancer Res*, 32(6):2347-2352.

Ni CM, Ling BY, Xu X, et al., 2020. CX3CR1 contributes to streptozotocin-induced mechanical allodynia in the mouse spinal cord. *J Zhejiang Univ-Sci B (Biomed & Biotechnol)*, 21(2):166-171. https://doi.org/10.1631/jzus.B1900439

Pankla R, Buddhisa S, Berry M, et al., 2009. Genomic transcriptional profiling identifies a candidate blood biomarker signature for the diagnosis of septicemic melioidosis. *Genome Biol*, 10(11):R127. https://doi.org/10.1186/gb-2009-10-11-r127

Patten M, Bünemann J, Thoma B, et al., 2002. Endotoxin induces desensitization of cardiac endothelin-1 receptor signaling by increased expression of RGS4 and RGS16. *Cardiovasc Res*, 53(1):156-164. https://doi.org/10.1016/S0008-6363(01)00443-6

Raspé C, Höcherl K, Rath S, et al., 2013. NF-κB-mediated inverse regulation of fractalkine and CX3CR1 during CLP-induced sepsis. *Cytokine*, 61(1):97-103. https://doi.org/10.1016/j.cyto.2012.08.034

Reinhart K, Bauer M, Riedemann NC, et al., 2012. New approaches to sepsis: molecular diagnostics and biomarkers. *Clin Microbiol Rev*, 25(4):609-634. https://doi.org/10.1128/Cmr.00016-12

Rhee C, Dantes R, Epstein L, et al., 2017. Incidence and trends of sepsis in US hospitals using clinical vs claims

data, 2009-2014. *JAMA*, 318(13):1241-1249. https://doi.org/10.1001/jama.2017.13836

Rhodes A, Evans LE, Alhazzani W, et al., 2017. Surviving sepsis campaign: international guidelines for management of sepsis and septic shock: 2016. *Intensive Care Med*, 43(3):304-377. https://doi.org/10.1007/s00134-017-4683-6

Rich JT, Neely JG, Paniello RC, et al., 2010. A practical guide to understanding Kaplan-Meier curves. *Otolaryngol Head Neck Surg*, 143(3):331-336. https://doi.org/10.1016/j.otohns.2010.05.007

Robin X, Turck N, Hainard A, et al., 2011. pROC: an open-source package for R and S+ to analyze and compare ROC curves. *BMC Bioinformatics*, 12:77. https://doi.org/10.1186/1471-2105-12-77

Ruan L, Chen GY, Liu Z, et al., 2018. The combination of procalcitonin and C-reactive protein or presepsin alone improves the accuracy of diagnosis of neonatal sepsis: a meta-analysis and systematic review. *Crit Care*, 22:316. https://doi.org/10.1186/s13054-018-2236-1

Schlatterer K, Beck C, Schoppmeier U, et al., 2021a. Acetate sensing by GPR43 alarms neutrophils and protects from severe sepsis. *Commun Biol*, 4:928. https://doi.org/10.1038/s42003-021-02427-0

Schlatterer K, Peschel A, Kretschmer D, 2021b. Short-chain fatty acid and FFAR2 activation—a new option for treating infections? *Front Cell Infect Microbiol*, 11:785833. https://doi.org/10.3389/fcimb.2021.785833

Scicluna BP, Klouwenberg PMCK, van Vught LA, et al., 2015. A molecular biomarker to diagnose community-acquired pneumonia on intensive care unit admission. *Am J Respir Crit Care Med*, 192(7):826-835. https://doi.org/10.1164/rccm.201502-0355OC

Silwal P, Kim JK, Kim YJ, et al., 2020. Mitochondrial reactive oxygen species: double-edged weapon in host defense and pathological inflammation during infection. *Front Immunol*, 11:1649. https://doi.org/10.3389/fimmu.2020.01649

Singer M, Deutschman CS, Seymour CW, et al., 2016. The third international consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA*, 315(8):801-810. https://doi.org/10.1001/jama.2016.0287

Subramanian A, Tamayo P, Mootha VK, et al., 2005. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci USA*, 102(43):15545-15550. https://doi.org/10.1073/pnas.0506580102

van der Poll T, van de Veerdonk FL, Scicluna BP, et al., 2017. The immunopathology of sepsis and potential therapeutic targets. *Nat Rev Immunol*, 17(7):407-420. https://doi.org/10.1038/nri.2017.36

Venet F, Monneret G, 2018. Advances in the understanding and treatment of sepsis-induced immunosuppression. *Nat Rev Nephrol*, 14(2):121-137. https://doi.org/10.1038/nrneph.2017.165

Venet F, Schilling J, Cazalis MA, et al., 2017. Modulation of LILRB2 protein and mRNA expressions in septic shock patients and after *ex vivo* lipopolysaccharide stimulation. *Hum Immunol*, 78(5-6):441-450. https://doi.org/10.1016/j.humimm.2017.03.010

Watanabe N, Suzuki Y, Inokuchi S, et al., 2016. Sepsis induces incomplete M2 phenotype polarization in peritoneal exudate cells in mice. *J Intensive Care*, 4:6. https://doi.org/10.1186/s40560-015-0124-1

Wedepohl S, Beceren-Braun F, Riese S, et al., 2012. L-Selectin – a dynamic regulator of leukocyte migration. *Eur J Cell Biol*, 91(4):257-264. https://doi.org/10.1016/j.ejcb.2011.02.007

Winters BD, Eberlein M, Leung J, et al., 2010. Long-term mortality and quality of life in sepsis: a systematic review. *Crit Care Med*, 38(5):1276-1283. https://doi.org/10.1097/CCM.0b013e3181d8cc1d

Xiao ZJ, Li JY, Yu Q, et al., 2021. An inflammatory response related gene signature associated with survival outcome and gemcitabine response in patients with pancreatic ductal adenocarcinoma. *Front Pharmacol*, 12:778294. https://doi.org/10.3389/fphar.2021.778294

Xu F, Zhou FC, 2020. Inhibition of microRNA-92a ameliorates lipopolysaccharide-induced endothelial barrier dysfunction by targeting ITGA5 through the PI3K/Akt signaling pathway in human pulmonary microvascular endothelial cells. *Int Immunopharmacol*, 78:106060. https://doi.org/10.1016/j.intimp.2019.106060

Zhong WW, Wang DJ, Yao B, et al., 2021. Integrative analysis of prognostic long non-coding RNAs with copy number variation in bladder cancer. *J Zhejiang Univ-Sci B (Biomed & Biotechnol)*, 22(8):664-681. https://doi.org/10.1631/jzus.B2000494

Zou WJ, Chen L, Mao WW, et al., 2021. Identification of inflammatory response-related gene signature associated with immune status and prognosis of lung adenocarcinoma. *Front Bioeng Biotechnol*, 9:772206. https://doi.org/10.3389/fbioe.2021.772206

Supplementary information

Tables S1−S5