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Original article

Identification of immune status subtypes and prognostic analysis of septic patients based on Th1/Th2 cytokine assays

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Abstract: Objective Sepsis patients exhibit diverse immune states, making it crucial to identify subtypes with distinct inflammatory profiles through Th1/Th2 cytokine data for personalized treatment and improved prognosis. **Methods** We retrieved data from sepsis patients who underwent Th1/Th2 cytokine testing in Nanfang Hospital, Southern Medical University from June 1, 2020, to February 1, 2022. An unsupervised K-means clustering method classified participants based on Th1/Th2 cytokine levels, with the primary outcome being the 7-day mortality rate post-ICU admission. Cox proportional hazards and Restricted Mean Survival Time (RMST) analyses were utilized to explore survival outcomes. **Results** A total of 321 sepsis patients were included. IL-6 (HR 1.69, 95%CI: 1.22, 2.34) and IL-10 (HR 1.81, 95% *CI*: 1.37, 2.40) emerged as independent predictors of 7-day mortality. Unsupervised K-means clustering revealed 3 inflammatory/immune subgroups: Cluster 1 (*n*=166, low inflammatory response), Cluster 2 (*n*=99, moderate inflammatory response with immune suppression), and Cluster 3 (*n*= 56, strong inflammatory and immune suppression). Compared to Cluster 1, Clusters 2 and 3 had higher 7-day mortality risks (14.4% *vs* 23.2%, HR=4.30, 95% *CI*: 1.51-12.26; 14.4% *vs* 35.7%, HR=7.32, 95% *CI*: 2.57-20.79). **Conclusion** Septic patients in a protective immune response state (Cluster 1) exhibit better short-term prognoses, suggesting the importance of understanding inflammatory/immune states for precise treatment and improved outcomes.

Keywords: Th1/Th2 cytokines; sepsis prognosis; K-means clustering; inflammatory/immune states

INTRODUCTION

Sepsis is characterized by life-threatening organ dysfunctions caused by a dysregulated host response to infection[1]. Globally, sepsis affects 27 to 30 million individuals and results in 7 to 9 million deaths annually [2-4]. Sepsis is associated with a high short-term mortality rate, ranging from 15% to 35%[2]. Immune response dysregulation is one of the common pathophysiological features of sepsis, manifested as cytokine storms, acquired immune suppression, metabolic disturbances, and multi-organ failure[5, 6]. Effective therapeutic approaches targeting dysregulated systemic inflammation in sepsis are currently lacking, partly due to the heterogeneity of individual immune conditions [6-8]. Timely identification of the inflammatory and immune statuses of septic patients is crucial for devising personalized therapeutic strategies and improving prognosis of the patients[9].

Cytokines, as key mediators of immune responses, play a crucial role in host defense against pathogens during sepsis. Sepsis-related pro-inflammatory cytokines consist primarily of interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ). Conversely, the anti-inflammatory cytokines mainly include IL-4 and IL-10 [10]. Most of previous studies assessed immune function in sepsis patients often by focusing on a single cytokine, which is often unable to accurately represent the immune status of septic due to its limited specificity and sensitivity for sepsis diagnosis and predicting patient outcomes[10]. Currently the drugs designed for targeted neutralization of a single proinflammatory cytokine (such as TNF-α, IL-1β and Toll-like receptors) failed to achieve satisfactory clinical outcomes[8, 10], possibly as a result of the intricate and time-dependent nature of the immune variables[7], suggesting the urgent need to identify distinct immune feature subgroups by comprehensively evaluating the clinical and host response data using clustering strategies. In addition, with the progression of sepsis, the pathogens evade host defenses to cause continuous cell damage and release of damage-associated molecular patterns (DAMPs), which

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activate pattern recognition receptors (PRRs) that detect microbial pathogen-associated molecular patterns (PAMPs) [11-13]. This mechanism initiates a cycle of sustained immune activation and functional impairment. Septic patients exhibit diverse immune responses, including protective reactions and combined conditions of inflammation and immunosuppression[11, 14, 15], thus creating a complex network of cytokines with varying peak time and effects. This further indicates that the identification of immune subpopulations based on dynamic cytokine profiles has significant diagnostic and prognostic value.

By analyzing genomic data from peripheral blood leukocytes, researchers classified pediatric and adult cohorts with septic shock into subgroups with distinct immune profiles, and found that the patients with immunosuppressive phenotypes had worse outcomes[16-18]. A secondary analysis of the VANISH randomized controlled trial additionally revealed a significant independent association between corticosteroid treatment given at the discretion of the treating physician and a higher mortality rate in the immunosuppressive phenotype subgroup[19]. This suggests that classification of immune subgroups could potentially inform treatment decisions. However, the high cost and time requirements for genomic testing limit its clinical use. Fang et al [7], by integrating plasma granulocyte-colony stimulating factor (G-CSF) levels, interleukin-10 (IL-10) levels, and expression levels of monocyte human leukocyte antigen-antigen D-related (HLA-DR), established an immune dysfunction scoring system for predicting 28-day mortality risk in septic patients, but its limited applicability and lack of external validation hindered its generalizability and ability to identify immune phenotype subgroups for targeted treatment. Tanaka et al[9] introduced an electrochemical point-to-point sensing device for multiplexed cytokine detection, known as the DETecT Sepsis sensor. This innovation enables rapid sepsis endotyping with small sample sizes. However, the clinical significance of multiplexed detection still needs validation in a larger patient cohort.

The primary aim of this study was to test the feasibility of identifying the subgroups with different sepsis-related inflammatory and immune statuses by utilizing early measurements of Th1/Th2 cytokines in ICU septic patients through the K-means clustering approach. We also sought to investigate the differences in early survival outcomes among these patient subgroups.

METHODS

Ethics

The study protocol was reviewed and approved by the Academic Committee and the Ethics Committee of Southern Medical University Nanfang Hospital (Approval No. NFEC-2022-008). Due to the retrospective nature of the study, the Ethics Committee waived the requirement for informed consent from the patients.

Patients

This study was a single-center, retrospective clinical study conducted among all septic patients admitted to the ICU of the Southern Medical University Nanfang Hospital with complete records of Th1/Th2 testing results (including IL-2, IL-4, IL-6, IL-10, TNF-α, and IFN-γ) between June 1, 2020, and February 1, 2022. Inclusion criteria: (1) age >18 years; (2) undergoing Th1/Th2 testing within 24 h after ICU admission; (3) meeting the diagnostic criteria described in the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3)[1]. The patients with ICU stay less than 24 h were excluded. For patients with multiple ICU admissions, only the results of the first ICU admission were analyzed.

Measurement of serum Th1/Th2 cytokine levels

Serum levels of IL-2, IL-4, IL-6, IL-10, TNF-α, and IFN-γ of the patients were measured using the Cytometric Beads Array (CBA) assay kit. The CBA technique is based on 6 distinct microbead sets, each with different fluorescence intensities and coated with antibodies specific to IL-2, IL-4, IL-6, IL-10, TNF-α, and IFN-γ proteins. The cytokine-capturing beads were mixed with the detection antibodies conjugated to phycoerythrin and then incubated with recombinant standards or test samples to form sandwich complexes. The sample data were then collected on a FACScalibur™ flow cytometer (Becton Dickinson, San Jose, CA, USA), and the results were generated in graphical and tabular formats using BD CBA software (BD Biosciences, San Jose, CA, USA). Six standard curves were obtained from a set of calibration standards, yielding 6 results for each test sample.

Data collection and clinical outcomes

The data of the key variables in the cytokine profiles of the septic patients were extracted, and the clinical data of each patient were retrospectively collected using a standardized form, including:

Demographic characteristics at ICU admission: age, gender, height, weight, underlying medical conditions, and comorbidities (including hypertension, diabetes, chronic heart failure, chronic liver disease, chronic obstructive pulmonary disease, chronic kidney dysfunction, malignancies, immune system disorders, trauma, acute respiratory distress syndrome), admission

type (surgical and non-surgical).

Cytokine features: including serum levels of IL-2, IL-4, IL-6, IL-10, TNF-α, and IFN-γ measured within 24 h of ICU admission.

Other laboratory test results: complete blood count (white blood cell count, WBC; neutrophil percentage, NEU% ; lymphocyte percentage, LYM% ; monocyte percentage, MONO% ; and platelet count, PLT); and infection markers (C-reactive protein, CRP; and procalcitonin, PCT) measured within 24 h of ICU admission.

Clinical interventions during ICU stay: use of antibiotics, glucocorticoids, duration of mechanical ventilation, renal replacement therapy, and use of vasoactive drugs and positive inotropic agents (including dopamine, dobutamine, epinephrine, norepinephrine, vasopressin, terlipressin).

Clinical scores: Acute Physiology and Chronic Health Evaluation II (APACHE II), Sequential Organ Failure Assessment (SOFA) score, and Glasgow Coma Scale.

Outcome events: The primary outcome was the survival status of patients at ICU day 7, and secondary outcomes included the survival status of patients at ICU day 14 and 28.

Statistical analysis

The category or binary variables were described using numbers (percentages), and non-normally distributed continuous variables were described using the median with interquartile range (IQR). To make the data more concentrated, a logarithmic transformation was applied to the IL-6 and IL-10 data. The correlation coefficients between serum cytokine levels were calculated using Spearman correlation analysis. We used Min-Max scaling on the serum cytokine levels to eliminate the effect of magnitude. We utilized Ordering Points to Identify the Clustering Structure (OPTICS) plots to determine the optimal clustering strategy[20]. Based on the OPTICS plots, we employed a partitioning approach to perform Consensus K-means clustering on the 6 cytokine variables[21]. To determine the optimal number of clusters in K-means clustering, we assessed the clear separation in the consensus matrix heatmap, the Silhouette Coefficient index (SC index), the Davies-Bouldin index (DB index), and the Calinski-Harabasz index (CH index). Additionally, we constructed an elbow plot. After obtaining classification labels for different clinical subtypes, we reduced the data to 3 dimensions using the t-distributed stochastic neighbor embedding (t-SNE) method for visualization. Violin plots were generated to depict the distribution of the studied cytokines across different clinical subtypes. Multiple comparisons of the cytokines between different subtypes were adjusted for using the False Discovery Rate (FDR) method.

We initially investigated the relationship between the levels of the 6 cytokines and the risk of death within 7, 14, and 28 days after ICU admission. In this study, the 6 cytokines were integrated into the model as continuous variables. We used Restricted Cubic Spline (RCS) plots based on the COX proportional hazards model to explore the dose-response relationship between the cytokine levels and the risk of death. We calculated the overall and nonlinear *P*-values. RCS curves were adjusted for potential confounders, including baseline demographic characteristics such as age, gender, and admission type. Three knots were placed in the spline function, with the lowest serum cytokine level as the reference value. To enhance the robustness, the levels of the 6 cytokines were integrated in the model using different approaches: as per standard deviation changes (i.e., per SD, with data transformation performed using Z-score standardization), and as 4 categories (categorized based on the quartile range of each cytokine level, with the lowest quartile as the reference value). Depending on the data type, Cox proportional hazards models were employed to calculate the HR and 95% *CI*. The Cox proportional hazards models were adjusted for confounding factors. Model 1 included adjustments for age, gender, and admission type. Model 2 was further adjusted for factors such as SOFA score, APACHE II score, and treatment history (including antibiotics, vasoactive drugs, dose of norepinephrine, positive inotropic agents, glucocorticoids, mechanical ventilation duration, and renal replacement therapy duration). Model 3 was additionally adjusted for comorbidities including hypertension, diabetes, chronic heart failure, chronic obstructive pulmonary disease, chronic kidney dysfunction, chronic liver dysfunction, malignancies, immune system disorders, trauma, and acute respiratory distress syndrome.

The relationship between unsupervised K-means clustering of the clinical subtypes and the risk of death within 7, 14, and 28 days after ICU admission was explored. The proportional hazards (PH) assumption was assessed using Kaplan-Meier (KM) curve visualization. For those meeting the PH assumption, Cox PH models were used to calculate the hazard ratios (HR) and the corresponding 95% confidence intervals (95% *CI*). For cases not meeting the PH assumption, restricted mean survival time (RMST) analysis was conducted. RMST is defined as the area under the KM survival curve at a predefined time point. The difference in RMST is interpreted as the difference in the area under the KM curves between two groups, indicating a reduction or an increase in survival time within the predefined time frame in the experimental group compared to the control group. The adjustment for confounding factors in different models was consistent with the correction method described above.

The R software, version 4.1.2 (R Project for Statistical Computing), was used for all statistical analyses. A two-sided *P* value <0.05 was considered to

indicate a statistically significant difference.

RESULTS

Patients

The primary analysis was conducted in a cohort of 321 septic patients with a median age of 58 (46, 69) years, including 242 male patients (75.4%; Fig.1). Tab.1 shows the baseline characteristics of the patients, test results of the key biomarkers, comorbidities and other clinical data. Death occurred in 20.9% of the patients during their ICU stay.

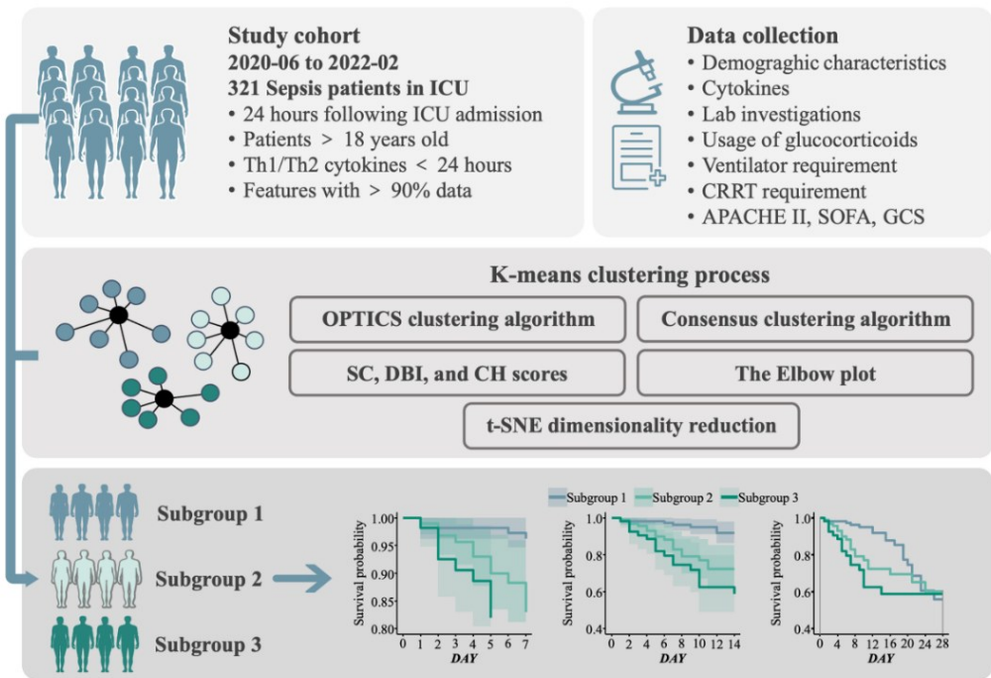

Fig. 1 Flow sheet of patient inclusion, data processing and subgroup identification. OPTICS: Ordering points to identify the clustering structure; SC: Silhouette score; DBI: Davies-Bouldin index; CH: Calinski-Harabasz score; t-SNE: t-distributed Stochastic Neighbor Embedding.

Inflammatory/immune status subgroups identified by unsupervised clustering of serum cytokine levels

The correlation coefficients among the cytokines ranged from 0.1 to 0.6. A heatmap depicting the pairwise correlations among the investigated parameters is shown in Fig.2A. The OPTICS plot exhibites a gradual increase in reachability distance (Fig.2B), suggesting a preference for clustering methods like consensus K-means clustering as the optimal statistical approach. We used 3 clusters as the optimal count, through a combination of consensus matrix heatmaps (Fig. 2C and Fig. 3), scores associated with clusters (including Silhouette score, Davies-Bouldin Score, and Calinski-Harabasz score; Fig. 2D), elbow plots (Fig. 2E), and considerations for clinical interpretability. We subsequently used the K-means clustering method to categorize all the 321 sepsis patients into 3 clusters (Fig. 2F). Tab. 2 shows the distribution of the cytokine levels following Min-Max scaling.

The characteristics of the patients across the 3 clusters are presented in Tab. 3. The violin plots show the cytokine profiles of the 3 clusters (Fig.4A). Serum IL-2 levels were significantly higher in Cluster 3 than in Cluster 1 and Cluster 2 (P_{FDR}<0.05) and did not differ significantly between the latter two clusters. Serum IL-4 levels were the lowest in Cluster 2 (P_{FDR}<0.05) and not significantly different between Cluster 1 and Cluster 3. Among the 3 clusters, log-IL-6 and log-IL-10 levels were the highest in Cluster 3 and the lowest in Cluster 2 (P_{FDR}<0.05). Serum TNF-α and IFN-γ levels were significantly higfher in Cluster 3 than in Cluster 2 (P_{FDR}< 0.05). Thus, Cluster 1 was characterized by a low inflammatory response and a mild anti-inflammatory response. Cluster 2 exhibited a moderate inflammatory response in a relative state of immune suppression. Cluster 3 showed intense inflammation and immune suppression.

Baseline characteristics of patient subgroups

The clusters showed variations in size (ranging from 17% to 51% of the cohort) and differences in disease severity (Fig. 4B), infection status (Fig. 4B), and treatment approaches (Fig. 4C). Further comprehensive information is presented in Tab. 3. Specifically, the

Tab.1 Baseline characteristics of all participants in this study (*n*=321)

Characteristics	Median [IQR]) or *n*(%)
Age (year)	58.0 [46.0, 69.0]
Male	242 (75.4%)
Admission type (surgery)	164 (51.1%)
SOFA score	7.0 [4.0, 10.0]
APACHE II score	19.0 [13.0, 26.0]
Hypertension	96 (29.9%)
Diabetes mellitus	72 (22.4%)
Congestive heart failure	36 (11.2%)
COPD	14 (4.4%)
CKD	29 (9.0%)
Chronic liver insufficiency	59 (18.4%)
Malignant tumors	99 (30.8%)
Immune system disorders	29 (9.0%)
Trauma	33 (10.3%)
ARDS	113 (35.2%)
Antibiotics	273 (85.0%)
Vasopressors	213 (66.4%)
Norepinephrine equivalent	0.09 [0.00, 0.24]
Positive inotropic drugs	27 (8.4%)
Glucocorticoid	32 (10.0%)
Ventilation time (h)	3.0 [1.0, 9.0]
RRT	91 (28.3%)
WBC ($\times10^9$/L)	12.09 [7.62, 16.65]
NEU (%)	86.9 [79.9, 92.3]
LYM (%)	6.8 [3.5, 11.5]
MONO (%)	4.8 [2.8, 7.2]
PLT ($\times10^9$/L)	139.0 [70.0, 220.0]
CRP (mg/L)	80.80 [37.77, 170.89]
PCT (ng/mL)	2.23 [0.40, 11.18]
IL-2 (pg/mL)	0.86 [0.55, 1.38]
IL-4 (pg/mL)	1.13 [0.72, 1.69]
Log(IL-6) (pg/mL)	5.01 [4.04, 6.63]
Log(IL-10) (pg/mL)	2.53 [1.78, 3.55]
TNF (pg/mL)	1.23 [0.87, 1.75]
IFN (pg/mL)	1.32 [0.85, 1.90]
Hospital mortality at ICU	67 (20.9%)

SOFA score: Sepsis-related organ failure assessment score (GCS is not included); APACHEII score: Acute Physiology and Chronic Health Enquiry II score; COPD: Chronic obstructive pulmonary disease; CKD: Chronic kidney disease; ARDS: Acute respiratory distress syndrome; RRT: Renal replacement therapy; IL-2: Interleukin-2; IL-4: Interleukin-4; IL-6: Interleukin-6; IL-10: Interleukin-10; TNF: Tumor necrosis factor; IFN: Interferon.

patients in Cluster 1 had lower disease severity with lower levels of aberrant laboratory test results. By comparison, the patients in Cluster 3 had a higher disease severity with higher proportions of patients requiring vasopressors, positive inotropic drugs, and renal replacement therapy. The patients in Cluster 2

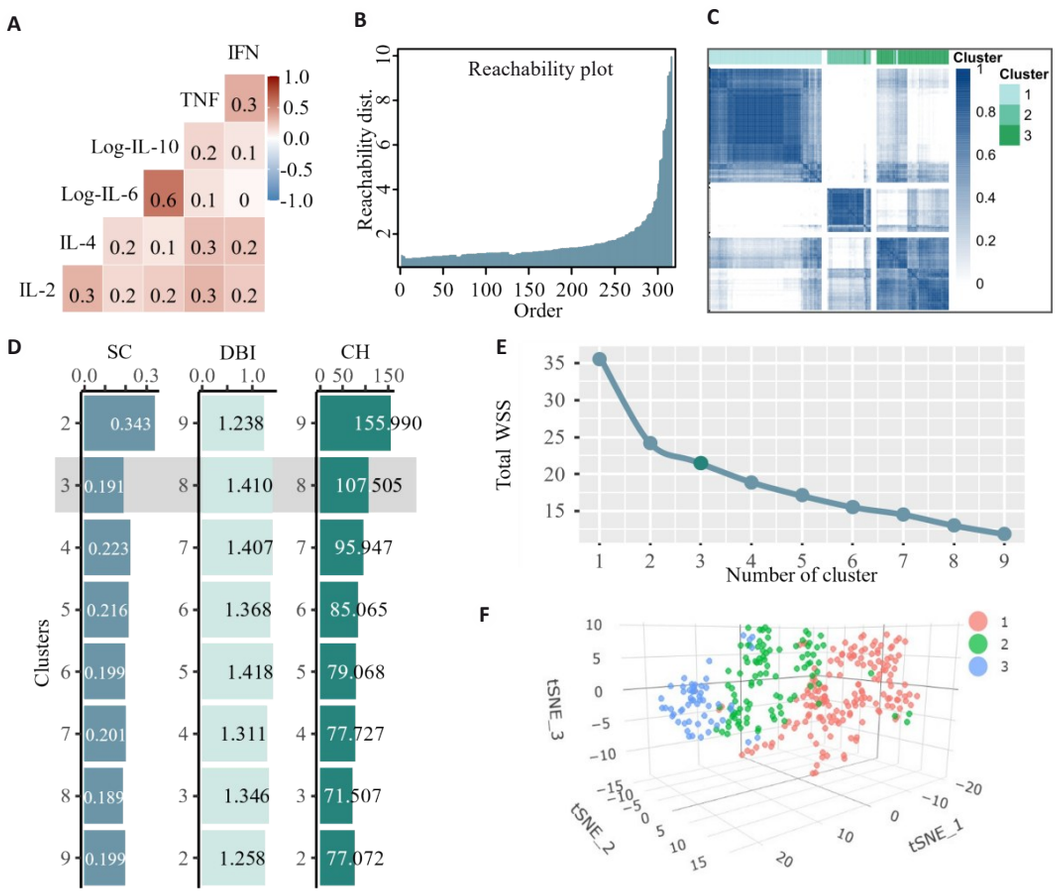

Fig. 2 Identification of the number of clusters of cytokine sub-phenotypes. **A**: Heat-map of pairwise correlations of the cytokines. **B**: OPTICS plot displaying a smooth rise in reachability distance. **C**: Heat map of the consensus matrix when the number of clusters was 3 (*k*=3). **D**: Histogram of SC, DBI, and CH values from *k*=2 to *k*=9 for clustering. E: Elbow plot showing the Total Within Cluster Sum of Squares (total WSS) for the number of clusters between 1 and 9. **F**: Visualization of K-means clustering results for 321 patients with sepsis based on cytokine profiles.

showed intermediate characterisitics between Cluster 1 and Cluster 3. Except for acute respiratory distress syndrome (ARDS), which was most pronounced in Cluster 3, the prevalence rates of comorbidities were largely simialr among the 3 clusters (Fig. 4C). Another noteworthy finding was the high rate of corticosteroid use in Cluster 3 (19.6% *vs* 7.8% in Cluster 1 and 8.1% in Cluster 2). The ICU mortality rates also differed among the 3 clusters (14% in Cluster 1, 23% in Cluster 2, and 36% in Cluster 3; Fig.4D).

Association between serum cytokine levels and risk of ICU mortality

Log-IL-6 showed a positive and non-linear correlation with the risk of mortality within 7 days after ICU admission ($P_{overall=}$0.01, $P_{non-linear}$=0.012; Fig. 5A and Tab. 4). Serum Log-IL-10 levels, on the other hand, demonstrated a positive linear dose-response relationship with the risk of mortality at 7 days after ICU admission ($P_{overall}$=0.001, $P_{non-linear}$=0.238). A notable trend observed was that in the majority of patients higher levels of IL-2, IL-4, TNF, and IFN were associated with increased risks of mortality within 7 days. These trends also apply to the survival outcomes at 14 and 28 days following ICU admission (Fig.6 and Tab.4). To enhance the robustness of these findings, we categorized cytokine levels into quartiles, and used the lowest quartile as the reference with subsequent adjustment for demographic factors, disease severity, treatment history, and comorbidities. This approach yielded consistent results with those obtained from the RCS analysis (Fig. 5B, Fig.7, and Tab.5). To ensure the reliability of the results, we conducted a sensitivity analysis of the relationship between the changes in cytokine levels by one standard deviation and the risk of mortality within 7, 14, and 28 days (Tab.6).

Association between serum cytokine-based inflammatory/immune status clusters and the risk of ICU mortality

We calculated the ICU in-hospital mortality risk scores for each cluster. As shown in Fig.8A, Cluster 3 had the highest risk scores and the greatest proportions of death

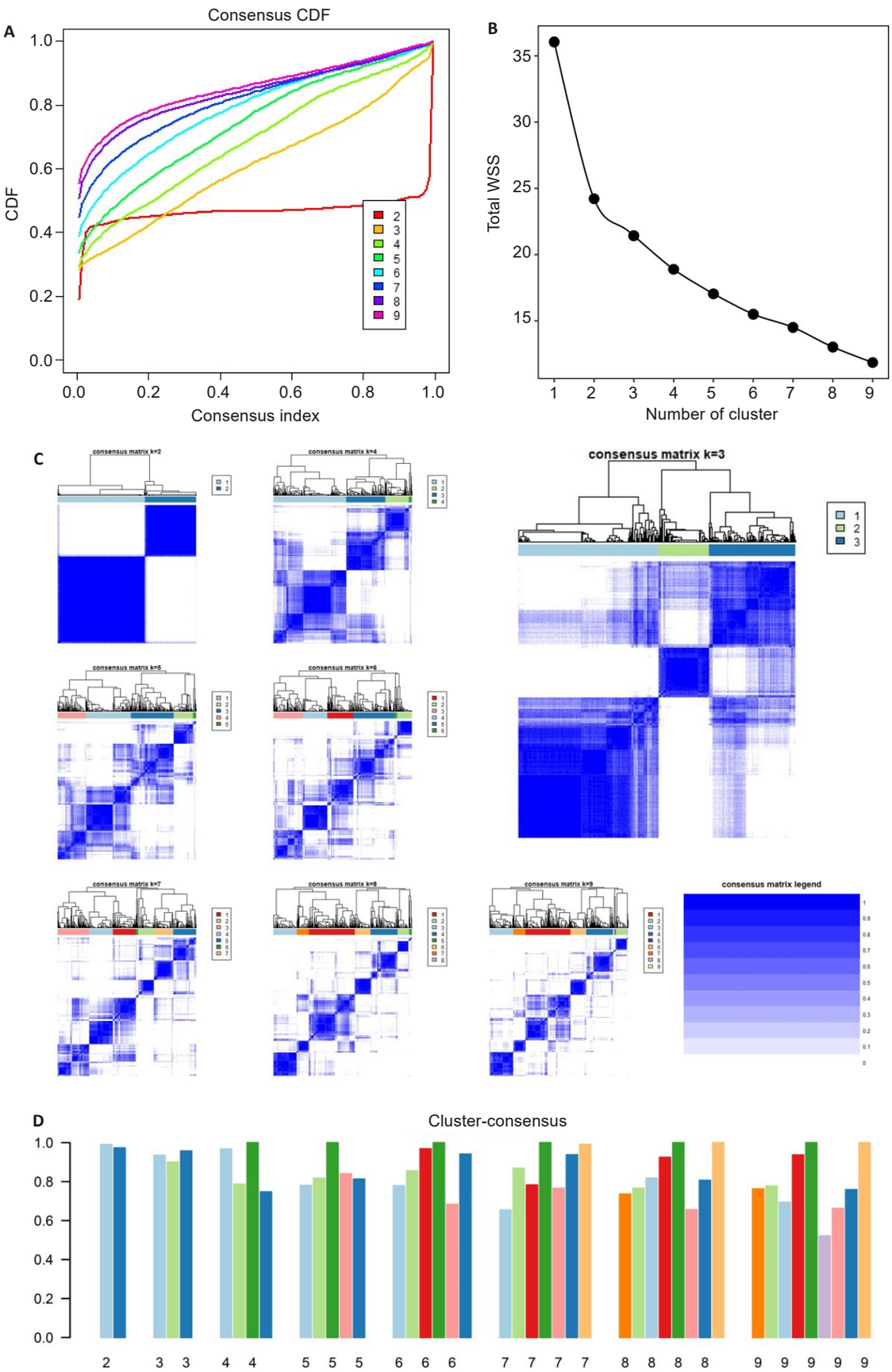

Fig. 3 Results of consensus clustering. The CDF graph shows the consensus distribution of each cluster (**A**). The delta area plot displays the relative change in the area under the CDF curve (**B**). The maximum change in the area occurs between *k*=2 and *k*=9 when the relative increase in the area becomes significantly smaller. As shown in the CM heat map (**C**), cluster 2 and cluster 3 identified by the K-means algorithm have clear boundaries, indicating good cluster stability in repeated iterations. The mean cluster consensus score was comparable between a scenario of 2 or 3 clusters (**D**).

Tab.2 Distributions of the studied cytokines after Min-Max scaling

Variable	25th percentile	Median	Mean	75th percentile
IL2	0.032	0.051	0.074	0.081
IL4	0.118	0.185	0.203	0.277
Log(IL-6)	0.370	0.474	0.505	0.648
Log(IL10)	0.243	0.336	0.366	0.464
TNF	0.048	0.068	0.088	0.097
IFN	0.013	0.020	0.038	0.028

at 7, 14, and 28 days. To further explore the relationship between exposure to different inflammatory/immune subgroups and the risks of death at 7, 14, and 28-days post K-means clustering, we employed the Cox regression model and conducted a Restricted Mean Time to Survival (RMST) analysis (Fig. 8B) while adjusting for confounding factors (Fig. 8C). In the unadjusted Cox model, Cluster 2 exhibited significant higher risks of mortality at 7 days (HR=4.41; 95% *CI*: 1.55-12.53) and 14 days (HR=4.01; 95% *CI*: 1.73, 9.30) than Cluster 1 ($P<0.05$). Following adjustment, only the risks of mortality at 7 days (HR=3.59; 95% *CI*: 1.21, 10.68) remained statistically significant between Cluster 1 and Cluster 2. In the unadjusted model, Cluster 3 had significantly higher risks of 7-day (HR=7.29; 95% *CI*: 2.57, 20.70) and 14-day mortality (HR=6.50; 95% *CI*: 2.81, 15.07) than Cluster 1 ($P<0.05$), but these differences were not statistically significant after adjustment for all the covariates. RMST analysis indicated that over the 28 days of ICU stay, Cluster 2 had a mean reduction in survival time by 2.49 days (95% *CI*: 0.67, 4.32) compared to Cluster 1, while Cluster 3 had a mean reduction by 4.51 days (95% *CI*: 1.24, 7.78); despite slight fluctuations in the values across the 3 models, these differences still remained statistically significant following covariate adjustment ($P<0.05$).

DISCUSSION

In this study we used the K-means clustering method to categorize 321 septic patients in ICU into 3 clusters based on their cytokine profiles indicative of their sepsis-associated inflammatory/immune states. In the 3 subgroups, the inflammatory response and anti-inflammatory reaction ranged from mild (Cluster 1) and moderate (Cluster 2) to severe (Cluster 3), and the latter two groups showed relative immune suppression (Cluster 2) and manifest immune suppression (Cluster 3). The patients in Cluster 1 exhibited the most favorable short-term prognosis, followed by those in Cluster 2, while in Cluster 3, the patients had the highest risk of short-term mortality. These results suggested that differentiating the inflammatory/immune status of critically ill patients can be instrumental for precision treatment and prognosis improvement.

Sepsis is characterized by an imbalanced immune response[5]. The dynamic pathological and physiological changes in the immune response of sepsis can be a potential reason for clustering and categorization. During the early stages of sepsis, the immune system deploys inflammatory, anti-inflammatory, and reparative mechanisms to eliminate pathogenic microorganisms and maintain local immune stability[11]. But with the disease progression, pathogens manage to elude the host's defense mechanisms to cause sustained injuries to the host cells and ongoing release of DAMPs, resulting in a complex, interconnected network system of pro-inflammatory and anti-inflammatory cytokines with varying effects and different onset and peak time. Identification of these heterogeneous immune feature Cluster from the dynamic cytokine profiles has significant diagnostic and prognostic value for sepsis.

We noted that the patients in Cluster 2, as compared with those in Cluster 1, exhibited elevated levels of the anti-inflammatory factor IL-10 (adjusted $P<0.001$), while some of the inflammatory cytokines (such as IL-2, TNF, and IFN) showed no significant changes. The cytokine profile of Cluster 2 indicates an anti-inflammatory or relatively immunosuppressive state, suggesting that the body might be in a state of compensatory anti-inflammatory response syndrome (CARS)[22, 23]. Numerous animal experiments suggested that a moderate increase in IL-10, indicative of a compensatory anti-inflammatory response, can be protective in sepsis[24-26], which is consistent with our finding that patients with moderate IL-10 levels did not have significantly increased mortality rate during ICU stay. For IL-10 levels in the second and third quartiles, the HR and 95% *CI* were 0.34 (0.04, 3.03) and 0.67 (0.11, 4.23), respectively. In Cluster 3, both the pro-inflammatory factors (IL-6, TNF, and IFN, etc.) and anti-inflammatory factors (IL-10 and IL-4) increased significantly, indicating a state of intense inflammation and immune suppression, known as mixed antagonist response syndrome (MARS)[27].

We further investigated the prognosis of septic

Tab.3 Baseline characteristics of 321 patients stratified by the 3 clusters of cytokines mixture

Characteristics	Cluster 1	Cluster 2	Cluster 3	*P*
n (%)	166 (51.7%)	99 (30.8%)	56 (17.4%)	
Age (year)	58.00 [45.25, 66.75]	57.00 [45.50, 69.00]	61.00 [50.00, 69.25]	0.617
Male	125 (75.3)	76 (76.8)	41 (73.2)	0.885
Admission type (surgery)	83 (50.0)	55 (55.6)	26 (46.4)	0.508
SOFA score	6.00 [4.00, 8.00]	8.00 [5.00, 11.00]	11.00 [8.75, 12.00]	<0.001
APACHE II score	18.00 [12.00, 23.00]	21.00 [14.00, 26.00]	26.50 [19.75, 32.25]	<0.001
Hypertension	53 (31.9%)	26 (26.3%)	17 (30.4%)	0.620
Diabetes mellitus	41 (24.7%)	21 (21.2%)	10 (17.9%)	0.536
Congestive heart failure	21 (12.7%)	8 (8.1%)	7 (12.5%)	0.493
COPD	8 (4.8%)	4 (4.0%)	2 (3.6%)	0.909
CKD	16 (9.6%)	9 (9.1%)	4 (7.1%)	0.853
Chronic liver insufficiency	32 (19.3%)	15 (15.2%)	12 (21.4%)	0.570
Malignant tumors	50 (30.1%)	32 (32.3%)	17 (30.4%)	0.928
Immune system disorders	16 (9.6%)	10 (10.1%)	3 (5.4%)	0.568
Trauma	18 (10.8%)	10 (10.1%)	5 (8.9%)	0.918
ARDS	45 (27.1%)	38 (38.4%)	30 (53.6%)	0.001
Antibiotics	144 (86.7%)	81 (81.8%)	48 (85.7%)	0.547
Vasopressors	86 (51.8%)	75 (75.8%)	52 (92.9%)	<0.001
Norepinephrine equivalent	0.03 [0.00, 0.16]	0.10 [0.01, 0.24]	0.22 [0.10, 0.60]	<0.001
Positive inotropic drugs	9 (5.4%)	8 (8.1%)	10 (17.9%)	0.015
Glucocorticoid	13 (7.8%)	8 (8.1%)	11 (19.6%)	0.029
Ventilation time (h)	3.00 [1.00, 8.75]	3.00 [1.00, 8.00]	6.00 [2.75, 10.00]	0.018
RRT	33 (19.9%)	30 (30.3%)	28 (50.0%)	<0.001
WBC ($\times10^9$/L)	11.29 [7.32, 15.16]	12.81 [8.68, 17.00]	14.18 [6.12, 20.75]	0.086
NEU (%)	83.55 [78.17, 89.77]	90.20 [84.15, 93.65]	90.80 [84.42, 93.93]	<0.001
LYM (%)	8.60 [4.70, 13.17]	4.90 [2.65, 9.30]	4.55 [3.15, 8.43]	<0.001
MONO (%)	5.40 [3.52, 8.10]	4.40 [2.65, 6.40]	3.50 [1.60, 4.80]	<0.001
PLT ($\times10^9$/L)	160.50 [96.25, 241.25]	126.00 [63.00, 203.00]	83.00 [47.00, 150.50]	<0.001
CRP (mg/L)	67.66 [26.70, 134.38]	90.88 [49.50, 182.36]	134.39 [63.26, 228.79]	<0.001
PCT (ng/mL)	1.15 [0.24, 4.20]	3.07 [0.52, 9.09]	23.38 [6.10, 68.22]	<0.001
IL-2 (pg/mL)	0.86 [0.52, 1.34]	0.77 [0.52, 1.25]	1.02 [0.67, 1.95]	0.027
IL-4 (pg/mL)	1.14 [0.75, 1.76]	0.96 [0.62, 1.41]	1.46 [1.07, 1.94]	0.003
Log (IL-6, pg/mL)	4.37 [3.46, 5.11]	5.44 [4.59, 6.30]	7.82 [7.10, 8.89]	<0.001
Log (IL-10, pg/mL)	1.81 [1.43, 2.19]	3.17 [2.82, 3.62]	4.86 [4.02, 5.41]	<0.001
TNF (pg/mL)	1.23 [0.85, 1.78]	1.12 [0.90, 1.51]	1.40 [0.95, 2.41]	0.021
IFN (pg/mL)	1.27 [0.89, 1.89]	1.18 [0.69, 1.67]	1.62 [1.11, 2.27]	0.005

The differences of continuous variables between groups were identified with univariate analysis, where comparisons of the categorical variables between groups were made using Chi-square test or Fisher's tests as appropriate. Cluster 1: Exposure group with a high inflammatory response and low anti-inflammatory response; Cluster 2: Exposure group with a moderate inflammatory response and high anti-inflammatory or immunosuppression; Cluster 3: Exposure group with a mixed antagonistic response.

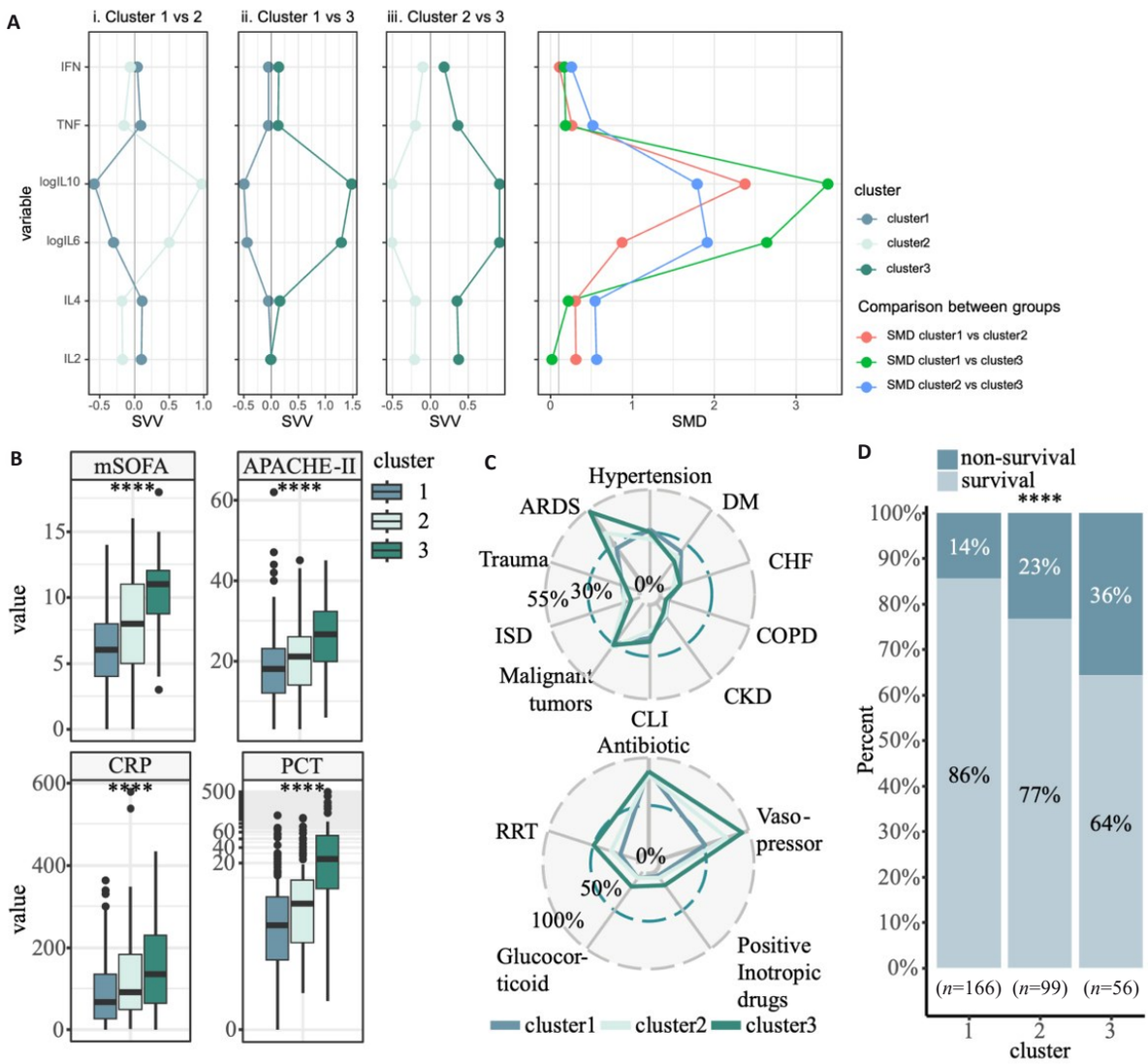

Fig.4 Clinical features of the 3 inflammatory subtypes of sepsis. **A**: Violin plots of serum cytokine levels in 3 inflammatory subtypes of sepsis. The *X*-axis represents the 3 subtypes, and the *Y*-axis represents serum cytokine levels. Comparisons between groups were corrected by false discovery rate (FDR). **B**: Box plots of mSOFA scores, APACHE-II scores, CRP levels, and PCT levels in the 3 inflammatory subtypes. **C**: Radar plots of the distribution of comorbidities and treatments in the 3 inflammatory subtypes (DM: Diabetes mellitus; CHF: Congestive heart failure; COPD: Chronic obstructive pulmonary disease; CKD: Chronic kidney disease; CLI: Chronic liver insufficiency; ISD: Immune system disorders; ARDS: Acute respiratory distress syndrome; RRT: Renal replacement therapy). **D**: Distribution of survival in the 3 inflammatory subtypes. ****P<0.0001.

patients in different clusters. The pateints in Cluster 3 had the worst prognosis, which was closely associated with their cytokine profile. Consistent with the results of previous cohort studies[28, 29], we found that elevated IL-10 levels was indicative of an immunosuppressive state, and hence a poorer prognosis. We also observed a significant positive correlation between serum IL-6 levels and the risk of death at 7, 14, and 28 days after ICU admission, which aligns with previous research findings[30, 31]. Immunosuppression, as seen in patients in Cluster 2 and 3 and despite its severity, was associated with a poorer prognosis. This observation is also supported by the study by Wong et al [16], who analyzed the blood leukocyte transcriptome data of 98 pediatric patients with septic shock using unsupervised clustering. They identified 3 subtypes of the septic patients, and among them, a subgroup characterized by reduced expressions of genes associated with adaptive immunity and glucocorticoid receptor signaling pathways had a significantly higher mortality rate. Davenport et al[18] identified two subgroups with distinct sepsis response feature by analyzing peripheral blood leukocyte genomic data of 265 patients with sepsis caused by community-acquired pneumonia, and the patients in the subgroup with gene expression profiles of an immunosuppressive phenotype showed a significantly higher mortality rate.

Accurate identification of the inflammatory/

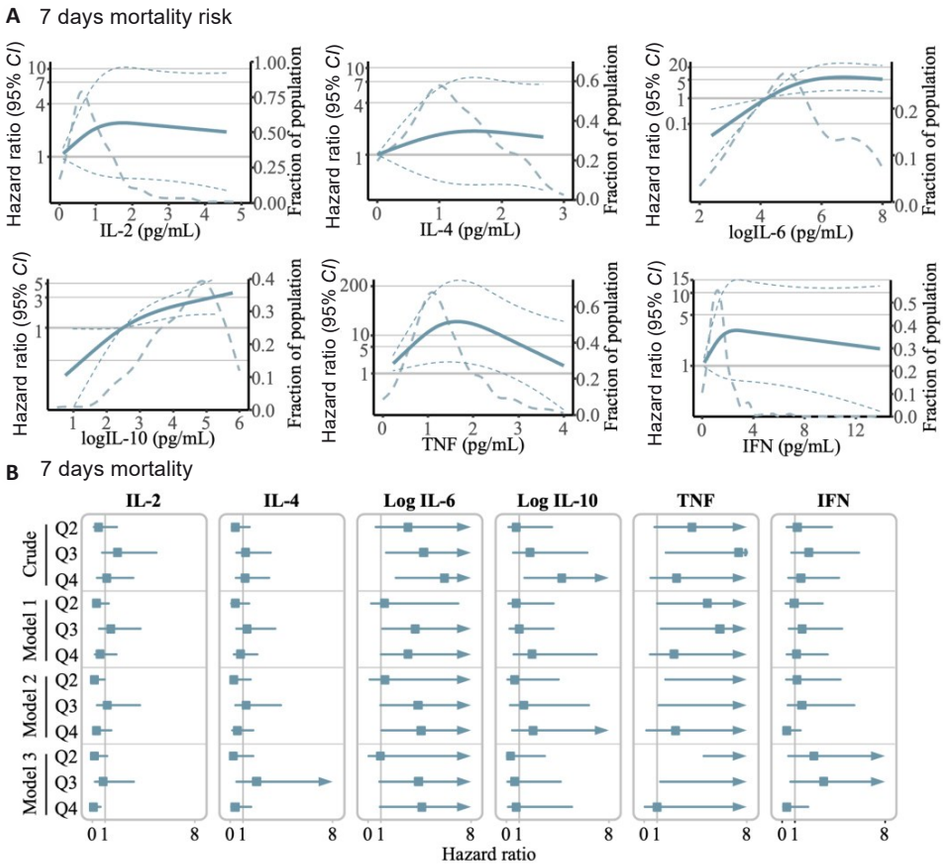

Fig. 5 Serum cytokine levels and risk of mortality in septic patients. **A**: Exposure-response relationship between circulating levels of each of the studied cytokines and the risk for 7 days mortality. The *Y*-axis represents the hazard ratio of the risk of death for a given value of circulating cytokine levels compared to the corresponding reference value (some values are converted to $\log_{10}$ on the *Y*-axis). The red dashed line indicates the 95% confidence interval, and the yellow dashed line the proportion of patients. **B**: Association between different serum cytokine levels and the risk of mortality within 7 days after ICU admission. Model 1 was adjusted for baseline age, sex, and admission type. Model 2 was additionally adjusted for SOFA score, APACHE II score, and relevant treatment history (use of antibiotics, vasopressors, norepinephrine equivalent, positive inotropic drugs, glucocorticoid, ventilation time, and RRT). Model 3 was further adjusted for history of chronic diseases (hypertension, diabetes, congestive heart failure, COPD, CKD, chronic liver insufficiency, malignant tumors, immune system disorders, trauma, ARDS, and CVD).

Tab.4 *P*-values of overall and non-linear dose-response relationships of the 6 cytokines with mortality risk

Cytokines	7-day mortality risk		14-day mortality risk		28-day mortality risk	
	Overall	Non-linear	Overall	Non-linear	Overall	Non-linear
IL-2	0.478	0.230	0.566	0.287	0.319	0.164
IL-4	0.695	0.475	0.867	0.757	0.912	0.847
Log(IL-6)	0.010	0.012	0.003	0.015	0.007	0.010
Log(IL-10)	0.001	0.238	<0.001	0.061	0.013	0.165
TNF	0.038	0.012	0.074	0.023	0.064	0.022
IFN	0.345	0.148	0.669	0.417	0.719	0.491

immune status in critically ill patients can be vital for clinical decisions on treatments. In an investigation using the VANISH cohort, Wong et al[19] reported that the effectiveness of corticosteroid therapy could vary across different immunophenotypes of sepsis, and corticosteroid therapy, decided by treating physicians, was correlated with higher mortality rates in patients with an immunosuppressive phenotype. We found that in Cluster 3, which had the highest mortality rate and a tendency towards MARS status, resported a

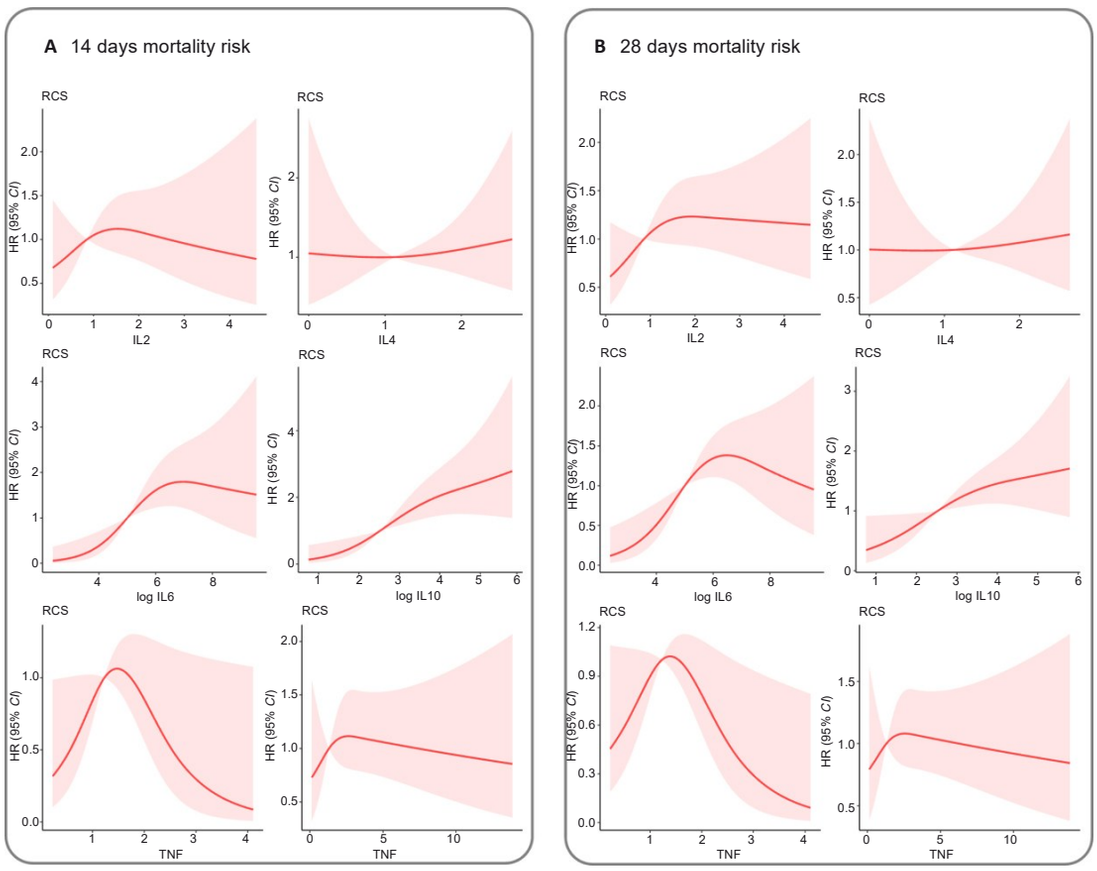

Fig.6 Exposure-response relationship between circulating levels of each of the studied cytokines and the risks of 14 day (**A**) and 28 day (**B**) mortality. The *Y*-axis represents the hazard ratios of mortality risk given the value of circulating cytokine levels compared to the corresponding reference value. The shaded areas indicate the 95 percent confidence intervals.

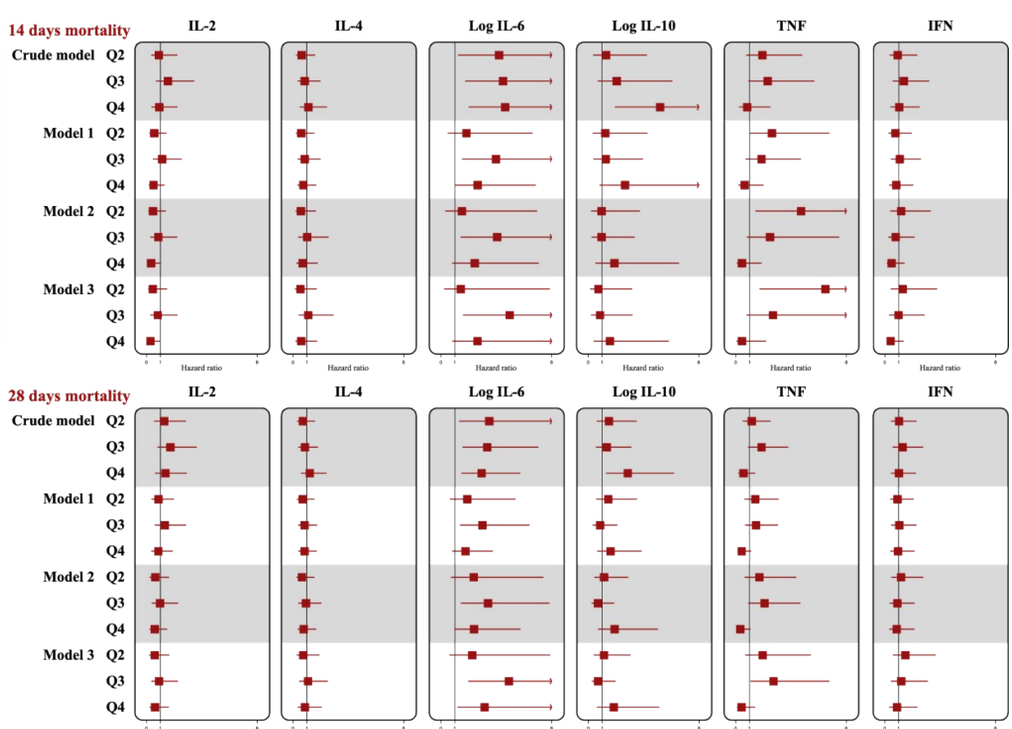

Fig. 7 Association between different serum cytokine levels and risk of death at 14 days and 28 days after ICU admission.

Tab.5 Crude and multi-variate adjusted hazard ratios (95% *CI*) of mortality risks in relation to levels of the 6 studied cytokines

Variable	Crude model	Model I[a]	Model 2[b]	Model 3[c]
7-day mortality risk				
IL-2				
Q1	1.00	1.00	1.00	1.00
Q2	0.51 (0.13, 1.95)	0.32 (0.08, 1.29)	0.19 (0.04, 0.96)	0.18 (0.03, 1.17)
Q3	1.97 (0.77, 4.99)	1.44 (0.55, 3.76)	1.16 (0.36, 3.74)	0.87 (0.23, 3.23)
Q4	1.12 (0.39, 3.21)	0.61 (0.20, 1.90)	0.35 (0.08, 1.48)	0.09 (0.01, 0.65)
IL-4				
Q1	1.00	1.00	1.00	1.00
Q2	0.40 (0.11, 1.52)	0.39 (0.10, 1.48)	0.29 (0.05, 1.60)	0.25 (0.04, 1.79)
Q3	1.21 (0.47, 3.14)	1.32 (0.50, 3.52)	1.25 (0.40, 3.95)	2.06 (0.50, 8.48)
Q4	1.17 (0.45, 3.04)	0.79 (0.30, 2.12)	0.57 (0.18, 1.81)	0.38 (0.09, 1.62)
Log (IL-6)				
Q1	1.00	1.00	1.00	1.00
Q2	3.09 (0.60, 15.91)	1.26 (0.23, 7.00)	1.31 (0.12, 14.46)	0.95 (0.04, 22.86)
Q3	**4.32 (1.37, 13.62)**	**3.66 (1.11, 12.04)**	3.88 (0.94, 15.97)	3.92 (0.86, 17.82)
Q4	**5.93 (2.16, 16.32)**	3.10 (0.99, 9.70)	**4.12 (1.02, 16.69)**	4.18 (0.90, 19.34)
Log (IL-10)				
Q1	1.00	1.00	1.00	1.00
Q2	0.79 (0.18, 3.55)	0.78 (0.17, 3.67)	0.67 (0.11, 4.07)	0.34 (0.04, 3.03)
Q3	1.85 (0.54, 6.32)	1.00 (0.27, 3.70)	1.37 (0.29, 6.44)	0.67 (0.11, 4.23)
Q4	**4.32 (1.43, 13.01)**	2.02 (0.58, 7.06)	2.10 (0.44, 10.10)	0.79 (0.12, 5.13)
TNF				
Q1	1.00	1.00	1.00	1.00
Q2	3.73 (0.79, 17.55)	**4.90 (1.00, 24.13)**	**23.56 (1.64, 338.28)**	**93.35 (4.62, 1885.71)**
Q3	**7.34 (1.67, 32.32)**	**5.89 (1.30, 26.74)**	**13.27 (1.12, 156.44)**	**16.65 (1.23, 225.81)**
Q4	2.52 (0.49, 13.01)	2.31 (0.43, 12.55)	2.44 (0.17, 35.54)	1.01 (0.04, 23.51)
IFN				
Q1	1.00	1.00	1.00	1.00
Q2	1.17 (0.36, 3.85)	0.93 (0.28, 3.14)	1.16 (0.30, 4.55)	2.47 (0.46, 13.41)
Q3	2.05 (0.7, 5.99)	1.54 (0.51, 4.67)	1.53 (0.42, 5.62)	3.22 (0.63, 16.49)
Q4	1.45 (0.48, 4.44)	1.12 (0.35, 3.56)	0.34 (0.08, 1.44)	0.34 (0.06, 2.03)
14-day mortality risk				
IL-2				
Q1	1.00	1.00	1.00	1.00
Q2	0.89 (0.36, 2.22)	0.56 (0.22, 1.43)	0.47 (0.16, 1.37)	0.45 (0.14, 1.46)
Q3	1.55 (0.71, 3.43)	1.13 (0.50, 2.53)	0.85 (0.32, 2.20)	0.81 (0.30, 2.23)
Q4	0.92 (0.38, 2.21)	0.50 (0.20, 1.29)	0.33 (0.11, 1.02)	0.28 (0.08, 0.95)
IL-4				
Q1	1.00	1.00	1.00	1.00
Q2	0.62 (0.24, 1.57)	0.59 (0.23, 1.52)	0.57 (0.20, 1.64)	0.53 (0.17, 1.69)
Q3	0.84 (0.36, 1.96)	0.84 (0.36, 1.97)	1.02 (0.41, 2.56)	1.10 (0.42, 2.91)
Q4	1.11 (0.51, 2.43)	0.74 (0.33, 1.67)	0.69 (0.27, 1.76)	0.59 (0.2,0 1.75)
Log (IL-6)				
Q1	1.00	1.00	1.00	1.00
Q2	4.20 (1.26, 13.98)	1.84 (0.51, 6.60)	1.51 (0.33, 6.92)	1.43 (0.26, 7.85)
Q3	**4.48 (1.80, 11.16)**	**3.97 (1.55, 10.15)**	**4.06 (1.45, 11.35)**	**4.97 (1.62, 15.27)**
Q4	**4.64 (2.03, 10.60)**	**2.65 (1.03, 6.83)**	2.44 (0.84, 7.06)	2.64 (0.85, 8.17)
Log (IL-10)				
Q1	1.00	1.00	1.00	1.00

Tab.5 (Continued)

Variable	Crude model	Model I[a]	Model 2[b]	Model 3[c]
Q2	1.29 (0.39, 4.23)	1.24 (0.36, 4.26)	0.97 (0.25, 3.72)	0.74 (0.17, 3.17)
Q3	2.07 (0.71, 6.06)	1.27 (0.41, 3.94)	0.97 (0.28, 3.35)	0.86 (0.23, 3.19)
Q4	**5.2 (1.96, 13.8)**	2.66 (0.87, 8.11)	1.90 (0.55, 6.55)	1.58 (0.43, 5.82)
TNF				
Q1	1.00	1.00	1.00	1.00
Q2	1.93 (0.78, 4.79)	**2.62 (1.02, 6.76)**	4.72 (1.44, 15.43)	**6.48 (1.75, 23.98)**
Q3	2.31 (0.94, 5.66)	1.86 (0.74, 4.69)	2.48 (0.83, 7.45)	2.69 (0.80, 9.01)
Q4	0.83 (0.28, 2.48)	0.64 (0.21, 1.98)	0.45 (0.11, 1.84)	0.45 (0.09, 2.18)
IFN				
Q1	1.00	1.00	1.00	1.00
Q2	0.92 (0.36, 2.32)	0.75 (0.29, 1.92)	1.17 (0.41, 3.30)	1.28 (0.44, 3.75)
Q3	1.36 (0.58, 3.18)	1.07 (0.45, 2.57)	0.77 (0.28, 2.12)	0.98 (0.33, 2.85)
Q4	1.03 (0.43, 2.50)	0.82 (0.33, 2.03)	0.48 (0.17, 1.40)	0.41 (0.13, 1.34)
28-day mortality risk				
IL-2				
Q1	1.00	1.00	1.00	1.00
Q2	1.27 (0.57, 2.84)	0.86 (0.38, 1.97)	0.63 (0.25, 1.60)	0.59 (0.21, 1.65)
Q3	1.72 (0.82, 3.61)	1.33 (0.62, 2.85)	0.97 (0.41, 2.27)	0.90 (0.36, 2.25)
Q4	1.37 (0.65, 2.89)	0.85 (0.38, 1.88)	0.60 (0.25, 1.46)	0.61 (0.23, 1.59)
IL-4				
Q1	1.00	1.00	1.00	1.00
Q2	0.71 (0.32, 1.57)	0.69 (0.31, 1.53)	0.64 (0.27, 1.53)	0.74 (0.29, 1.89)
Q3	0.86 (0.41, 1.78)	0.83 (0.40, 1.73)	0.93 (0.42, 2.03)	1.08 (0.47, 2.51)
Q4	1.21 (0.61, 2.40)	0.83 (0.41, 1.69)	0.77 (0.36, 1.66)	0.86 (0.36, 2.05)
Log (IL-6)				
Q1	1.00	1.00	1.00	1.00
Q2	3.49 (1.34, 9.10)	1.90 (0.67, 5.38)	2.37 (0.76, 7.39)	2.26 (0.65, 7.88)
Q3	**3.34 (1.59, 7.02)**	3.00 (1.41, 6.38)	**3.40 (1.47, 7.82)**	**4.91 (2.00, 12.03)**
Q4	**2.94 (1.51, 5.71)**	1.77 (0.84, 3.73)	**2.39 (1.00, 5.72)**	**3.15 (1.23, 8.08)**
Log (IL-10)				
Q1	1.00	1.00	1.00	1.00
Q2	1.48 (0.63, 3.47)	1.43 (0.59, 3.49)	1.13 (0.45, 2.86)	1.12 (0.41, 3.03)
Q3	1.32 (0.56, 3.09)	0.84 (0.34, 2.07)	0.69 (0.26, 1.84)	0.70 (0.26, 1.94)
Q4	**2.84 (1.30, 6.17)**	1.59 (0.66, 3.83)	1.89 (0.71, 5.01)	1.83 (0.65, 5.10)
TNF				
Q1	1.00	1.00	1.00	1.00
Q2	1.17 (0.55, 2.51)	1.42 (0.65, 3.10)	1.72 (0.68, 4.36)	1.96 (0.71, 5.42)
Q3	1.87 (0.93, 3.79)	1.48 (0.71, 3.06)	2.09 (0.94, 4.66)	**2.74 (1.11, 6.76)**
Q4	0.57 (0.23, 1.40)	0.44 (0.18, 1.12)	0.34 (0.11, 1.06)	0.43 (0.13, 1.39)
IFN				
Q1	1.00	1.00	1.00	1.00
Q2	1.03 (0.47, 2.28)	0.92 (0.41, 2.07)	1.17 (0.50, 2.76)	1.49 (0.61, 3.66)
Q3	1.28 (0.60, 2.75)	1.05 (0.49, 2.28)	0.91 (0.39, 2.13)	1.19 (0.46, 3.09)
Q4	1.02 (0.47, 2.24)	0.96 (0.43, 2.14)	0.86 (0.35, 2.12)	0.88 (0.33, 2.34)

[a]Model 1 was adjusted for baseline age, sex, and admission type; [b]Model 2 was additionally adjusted for SOFA score, APACHE II score, and the relevant treatment history (use of antibiotics, vasopressors, norepinephrine equivalent, positive inotropic drugs, glucocorticoid, ventilation time, and RRT). [c]Model 3 was further adjusted for the history of chronic diseases (hypertension, diabetes, congestive heart failure, COPD, CKD, chronic liver insufficiency, malignant tumors, immune system disorders, trauma, ARDS, and CVD). Bold values are statistically significant.

Tab.6 Crude and multi-variate adjusted hazard ratios (95% *CIs*) of the 7-, 14-, and 28-day mortality risks in relation to the per SD increase in the six studied cytokines

Variables	Crude model	Model I	Model 2	Model 3
7-days mortality risk				
IL2	1.02 (0.71, 1.46)	0.92 (0.55, 1.53)	0.95 (0.45, 2.00)	0.36 (0.12, 1.11)
IL4	1.09 (0.77, 1.56)	1.02 (0.71, 1.48)	0.85 (0.56, 1.30)	0.75 (0.47, 1.22)
Log-IL6	**1.69 (1.22, 2.34)**	1.42 (0.97, 2.08)	**1.91 (1.08, 3.37)**	**2.00 (1.03, 3.91)**
Log-IL10	**1.81 (1.37, 2.40)**	**1.41 (1.00, 1.99)**	1.45 (0.90, 2.33)	1.20 (0.70, 2.06)
TNF	0.94 (0.57, 1.56)	0.83 (0.45, 1.55)	0.47 (0.20, 1.09)	**0.34 (0.13, 0.92)**
IFN	0.87 (0.50, 1.53)	0.74 (0.41, 1.35)	0.56 (0.21, 1.49)	**0.09 (0.01, 0.63)**
14-day mortality risk				
IL2	0.95 (0.66, 1.38)	0.79 (0.46, 1.34)	0.63 (0.31, 1.25)	0.46 (0.20, 1.03)
IL4	1.06 (0.79, 1.44)	1.00 (0.72, 1.38)	0.98 (0.71, 1.36)	0.99 (0.70, 1.41)
Log-IL6	**1.62 (1.24, 2.12)**	**1.39 (1.01, 1.91)**	1.37 (0.92, 2.04)	1.45 (0.94, 2.22)
Log-IL10	**1.71 (1.36, 2.15)**	1.30 (0.97, 1.73)	1.40 (0.93, 2.10)	1.35 (0.89, 2.05)
TNF	0.79 (0.46, 1.37)	0.61 (0.33, 1.12)	**0.35 (0.16, 0.77)**	**0.30 (0.13, 0.71)**
IFN	0.91 (0.61, 1.37)	0.75 (0.49, 1.16)	0.57 (0.31, 1.03)	**0.41 (0.20, 0.84)**
28-day mortality risk				
IL2	1.08 (0.84, 1.39)	1.02 (0.74, 1.39)	0.97 (0.68, 1.38)	1.00 (0.66, 1.53)
IL4	1.05 (0.81, 1.37)	0.98 (0.74, 1.30)	0.97 (0.73, 1.28)	1.01 (0.75, 1.36)
Log-IL6	**1.36 (1.08, 1.72)**	1.16 (0.89, 1.51)	1.26 (0.90, 1.76)	1.38 (0.98, 1.96)
Log-IL10	**1.37 (1.11, 1.69)**	1.07 (0.83, 1.38)	1.34 (0.93, 1.95)	1.32 (0.91, 1.92)
TNF	0.73 (0.44, 1.20)	0.56 (0.32, 0.98)	**0.43 (0.23, 0.82)**	**0.45 (0.23, 0.86)**
IFN	0.91 (0.62, 1.33)	0.81 (0.54, 1.20)	0.69 (0.42, 1.13)	**0.57 (0.33, 0.98)**

'Log' indicates logarithmic transformation.Bold indicates statistical significance.

corticosteroid usage rate of 19.6%, signifianylty higher than the rates in the other two subgroups (P=0.029).

This study has some limitations. First, we only examined cytokine profiles of the patients within the first 24 h after ICU admission, and did not consider their possible changes prior to the admission. As such, future investigations are warranted to examine the cytokine profiles of septic patients in pre-hospital or emergency department settings to capture a more comprehensive picture of the cytokines. Second, we only analyzed cross-sections of the cytokine profiles and failed to explore their variations over time that may potentially impact the patients′ outcome. Third, we identified 3 subgroups with distinct immune features, but the relative sample size did not allow further discernment of more homogeneous subsets within a particular subgroup. Lastly, we did not analyze the differences in responses to immune-modulatory treatments across the 3 subgroups, which shall be addressed in future randomized controlled studies based on identification of specific subgroups.

In conclusion, septic patients in a protective immune response state (Cluster 1) show better short-term prognosis, while those with CARS (Cluster 2) and MARS (Cluster 3) are exposed to significantly increased risks of short-term mortality. This underscores the significance of identifying the inflammatory/immune states in critically ill patients to enable precision treatment and improve survial outcomes of septic patients.

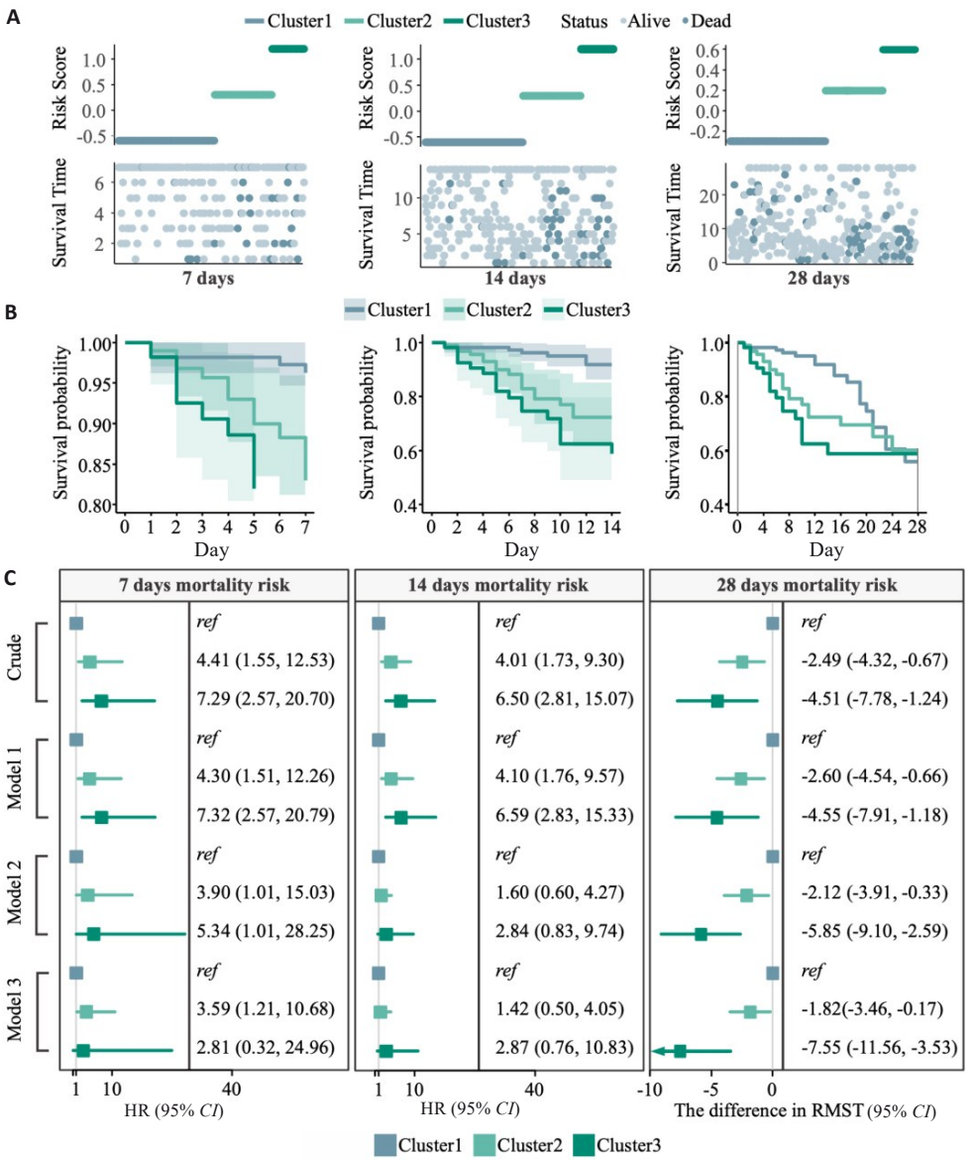

Fig. 8 Association of cytokine inflammatory subtypes with ICU mortality risk. **A**: Risk score plot. **B**: Kaplan-Meier analysis of different cytokine inflammatory subtypes on days 7, 14, and 28 in septic patients in the ICU. **C**: Forest plots of 7-day, 14-day, and 28-day mortality risk for the 3 inflammatory subtypes. The difference in RMST (95% *CI*) was calculated using the difference in restricted mean survival time between the two groups ($RMST_{cluster2}$-$RMST_{cluster1}$, or $RMST_{cluster3}$-$RMST_{cluster1}$), implying a decrease in survival in the other clustes relative to Cluster 1.

Declaration of interests: The authors declare no competing interests.

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基于 Th1/Th2 细胞因子检测的脓毒症免疫状态分型及预后分析：一项回顾性研究

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摘要：目的 通过Th1/Th2细胞因子数据识别具有不同炎症特征的脓毒症亚型以制定个性化治疗方案，改善患者预后。**方法** 在南方医科大学南方医院数据库中检索2020年6月1日~2022年2月1日期间接受Th1/Th2细胞因子检测的脓毒症患者数据。通过无监督K-均值聚类方法，根据Th1/Th2细胞因子水平对研究对象进行分类，主要研究终点为入ICU后7 d死亡率。采用Cox比例风险模型和限制平均生存时间（RMST）分析不同类型患者的生存结局。**结果** 共纳入321例脓毒症患者。IL-6（HR=1.69，95% *CI*：1.22~2.34）和IL-10（HR=1.81，95% *CI*：1.37~2.40）被确定为患者入ICU后7 d内死亡率的独立预测因子。无监督K-均值聚类分析识别出3种炎症/免疫亚组：亚组1（*n*=166，低炎症反应）、亚组2（*n*=99，中度炎症反应伴免疫抑制）、亚组3（*n*=56，强烈炎症和免疫抑制）。与亚组1相比，亚组2和亚组3的患者入ICU后7 d内死亡风险更高（14.4% *vs* 23.2%，HR=4.30，95% *CI*：1.51~12.26；14.4% *vs* 35.7%，HR=7.32，95% *CI*：2.57~20.79）。**结论** 处于保护性免疫反应状态（亚组1）的脓毒症患者短期预后较好，提示准确识别患者的炎症/免疫状态对精准治疗和改善结局的重要性。

关键词：Th1/Th2细胞因子；脓毒症；预后；K-均值聚类；炎症/免疫状态

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