

# Immune Subtypes in Sepsis: A Retrospective Cohort Study Utilizing Clustering Methodology

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**Background:** Sepsis is a heterogeneous clinical syndrome. Identifying distinct clinical phenotypes may enable more targeted therapeutic interventions and improve patient care.

**Objective:** This study aims to use clustering analysis techniques to identify different immune subtypes in sepsis patients and explore their clinical relevance and prognosis.

**Methods:** The study included 236 patients from the EICU at Shanghai Tenth People's Hospital, who met the Sepsis 3.0 diagnostic criteria. Blood samples were collected to measure lymphocyte subsets and cytokine levels, along with demographic and clinical data. K-means clustering analysis was used to categorize patients into three groups based on immune and inflammatory markers.

**Results:** Three immune subtypes were identified: the high immune activation subtype (Cluster 1), characterized by high levels of CRP and WBC, high levels of T cells, NK cells, and B cells, and low levels of IL-6, IL-8, and IL-10; the moderate immune activation subtype (Cluster 2), characterized by moderate levels of CRP, WBC, T cells, NK cells, B cells, IL-6, IL-8, and IL-10; and the high inflammation and immune suppression subtype (Cluster 3), characterized by very high levels of IL-6, IL-8, and IL-10, low levels of T cells, NK cells, and B cells, and relatively lower CRP levels. Patients in Cluster 3 had a significantly increased 28-day mortality risk compared to those in Cluster 1 (HR = 21.65, 95% CI: 7.46–62.87,  $p < 0.001$ ). Kaplan-Meier survival curves showed the lowest survival rates for Cluster 3 and the highest for Cluster 1, with the differences among the three groups being highly statistically significant ( $p < 0.0001$ ).

**Conclusion:** This study identified three immune subtypes of sepsis that are significantly associated with clinical outcomes. These findings provide evidence for personalized treatment strategies to improve patient outcomes.

**Keywords:** sepsis, immune subtypes, clustering analysis, prognosis, cytokines

## Introduction

Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection.<sup>1,2</sup> It poses a significant challenge to global public health, affecting approximately 19 million people worldwide annually, creating a substantial societal burden.<sup>3,4</sup> Despite significant improvements in treatment success rates due to the widespread adoption of clinical guidelines and a deeper understanding of sepsis pathophysiology, targeted therapies for immune dysregulation in sepsis patients have frequently failed. The primary reason for these failures is the significant heterogeneity of sepsis, which complicates diagnosis and treatment. Standardized treatment protocols often fail to meet the needs of all patients, highlighting the urgent need to explore sepsis subtypes and move towards personalized medicine.

Recent studies analyzing clinical manifestations and biomarkers (such as plasma proteins and genetic expression) have successfully identified different sepsis subtypes.<sup>5–7</sup> These subtypes not only reveal biological differences among patients but also provide a scientific basis for developing targeted therapeutic strategies. During the progression of sepsis,

the host's immune system triggers a strong inflammatory response to combat pathogens, involving numerous inflammatory mediators and cytokines, such as interleukin-6 (IL-6), interleukin-8 (IL-8), and interleukin-10 (IL-10).<sup>8</sup> Monitoring white blood cell count, C-reactive protein (CRP) levels, and the aforementioned cytokines allows clinicians to assess the severity of inflammation and immune status, guiding treatment decisions.<sup>9</sup> However, single inflammatory markers cannot comprehensively understand and assess the immune status of sepsis patients, making it challenging to effectively guide immunomodulatory therapies, which is a critical issue in clinical practice.

Existing studies have made significant progress in identifying sepsis subtypes, but some limitations remain. For example, most studies focus on single biomarkers or clinical features, lacking comprehensive analysis of multidimensional data.<sup>10</sup> Additionally, few studies have utilized unsupervised learning methods for clustering analysis of large-scale data to identify clinically significant immune subtypes.

This study plans to use clustering analysis techniques from unsupervised learning to identify different immune subtypes in sepsis patients by analyzing immune biomarker data in detail and exploring their clinical relevance and prognosis. This research effort will provide a scientific basis for personalized treatment of sepsis and optimize therapeutic strategies to improve clinical outcomes for patients.

## Method

### Study Design

The study was carried out in the Emergency Intensive Care Unit (EICU) at Shanghai Tenth People's Hospital, which is affiliated with Tongji University. It obtained approval from the hospital's ethics committee with the approval number 23K65 and was registered with the Chinese Clinical Trial Registry, with the registration number ChiCTR2300077055 accessible at [www.chictr.org.cn](http://www.chictr.org.cn). This research adheres to the Declaration of Helsinki and has been approved by the hospital's ethics committee.

### Participants

All participants provided written informed consent. Inclusion criteria were: a) meeting Sepsis 3.0 diagnostic criteria, b) age 18–85 years, and c) hospitalization for more than 24 hours. Exclusion criteria included: a) end-stage chronic diseases, b) pregnancy, c) solid or hematologic malignancies, and d) use of immunosuppressants for more than 3 months. A total of 236 sepsis patients were enrolled from August 2023 to July 2024.

### Data Collection

Blood samples were collected in EDTA tubes and serum collection tubes. Lymphocyte subsets (including total T cells, B cells, and natural killer cells) in whole blood samples were detected using kits from Jiangxi Saien Biotechnology Co., Ltd. and the Multitest TM 6-color TBNK reagent kit from BD Biosciences, USA. Cytokine levels (IL-6, IL-8, IL-10) in serum samples were measured using the FACS CantoII flow cytometer from BD Biosciences, USA. Demographic information, medical history, routine laboratory results, ICU stay duration, in-hospital mortality, and 28-day mortality data were also collected. Missing data for patients were less than 5% and were imputed using the mean values of the respective variables.

### Data Sharing Statement

The authors intend to share individual deidentified participant data that underlie the reported results. The data will include clinical measurements, laboratory values, and outcome data. The data will be available through direct request to the corresponding author for researchers who provide a methodologically sound proposal. Data will be available beginning 6 months and ending 24 months following article publication.

### Statistical Analysis

Statistical analyses were performed using R version 4.0.3. Continuous variables were expressed as mean  $\pm$  SD for normally distributed data and as median (IQR) for non-normally distributed data. Categorical variables were presented as frequencies and percentages. Normality was assessed using the Kolmogorov–Smirnov test. Group comparisons used the

Student's *t*-test for normally distributed continuous variables, the Mann–Whitney *U*-test for non-normally distributed continuous variables, and the chi-square or Fisher's exact test for categorical variables. K-means clustering was applied to standardize immune and inflammatory markers (CRP, WBC, T cells, NK cells, B cells, IL-6, IL-8, IL-10). The optimal number of clusters was determined using the elbow method and silhouette analysis.

Survival analysis was performed using Kaplan-Meier curves, with differences between groups assessed by the Log rank test. Cox regression models (univariate and multivariate) evaluated the association between immune subtypes and 28-day mortality, reporting hazard ratios (HRs) and 95% confidence intervals (CIs). Multivariate Cox models adjusted for confounders identified in univariate analysis, including age, gender, comorbidities (coronary artery disease, heart failure, chronic kidney disease), and severity scores (SOFA, APACHE II). The proportional hazards assumption was tested with Schoenfeld residuals. A two-sided *p*-value < 0.05 was considered statistically significant.

## Result

### Patient Characteristics

This study evaluated 236 sepsis patients, with 167 survivors and 69 non-survivors within 28 days. Non-survivors were older ( $71.4 \pm 10.0$  years) compared to survivors ( $66.6 \pm 14.2$  years,  $p = 0.011$ ), [Table 1](#). Among the entire cohort, gender and hypertension showed no significant survival impact. Non-survivors had higher rates of coronary heart disease (37.7% vs 18%,  $p = 0.001$ ), heart failure (18.8% vs 8.4%,  $p = 0.022$ ), and chronic kidney disease (18.8% vs 7.8%,  $p = 0.014$ ). Significant clinical differences included higher SOFA and APACHE II scores, and increased incidence of septic shock in non-survivors (55.1% vs 15%,  $p < 0.001$ ). Laboratory findings indicated that non-survivors had lower platelet counts, and prolonged prothrombin and partial thromboplastin times (all  $p < 0.001$ ). Furthermore, non-survivors presented with significantly higher levels of IL-6, IL-8, IL-10, ALT, AST, BUN, and BNP, along with lower albumin levels and reduced counts of T cells, NK cells, and B cells (all  $p < 0.001$ ). D-dimer levels were also elevated in non-survivors ( $p < 0.001$ ), while CRP levels and white blood cell counts did not differ significantly between groups.

### Cluster Analysis

Key inflammatory and immune parameters (CRP, WBC, T cells, NK cells, B cells, IL-6, IL-8, IL-10) were selected for cluster analysis. Data were standardized using a standardization function to achieve a mean of 0 and a standard deviation of 1. The optimal number of clusters was determined using the elbow method and silhouette coefficient method. The elbow method plotted the within-cluster sum of squares (WSS) against the number of clusters, identifying the “elbow point” where the rate of decrease slowed. The silhouette coefficient measured how similar an object was to its own cluster compared to other clusters. Based on these methods, three clusters were determined to be optimal. Subsequently, K-means clustering was employed to assign patients to one of three clusters based on their immune and inflammatory characteristics, as shown in [Figure 1](#). [Figure 2](#) illustrates the distribution of various clinical indicators across three clusters of sepsis patients:

#### Cluster 1 - High Immune Activation Subtype

Characterized by high levels of CRP and WBC, high levels of T cells, NK cells, and B cells, and low levels of cytokines IL-6, IL-8, and IL-10.

#### Cluster 2 - Moderate Immune Activation Subtype

Characterized by moderate levels of CRP and WBC, moderate levels of T cells, NK cells, and B cells, and moderate levels of cytokines IL-6, IL-8, and IL-10.

#### Cluster 3 - High Inflammation and Immunosuppression Subtype

Characterized by very high levels of cytokines IL-6, IL-8, and IL-10, low levels of T cells, NK cells, and B cells, and relatively lower CRP levels.

**Table 1** Baseline Characteristics of the Study Population

Variables	Total (n = 236)	Survivors (n = 167)	Non-survivors (n = 69)	p
Demographic Information				
Age	68.0 ± 13.3	66.6 ± 14.2	71.4 ± 10.0	0.011
Male	151 (64.0)	106 (63.5)	45 (65.2)	0.800
Hypertension	136 (57.6)	93 (55.7)	43 (62.3)	0.348
Diabetes Mellitus	83 (35.2)	54 (32.3)	29 (42)	0.156
Coronary Heart Disease	56 (23.7)	30 (18)	26 (37.7)	0.001
Heart Failure	27 (11.4)	14 (8.4)	13 (18.8)	0.022
Chronic Kidney Disease	26 (11.0)	13 (7.8)	13 (18.8)	0.014
COPD	45 (19.1)	32 (19.2)	13 (18.8)	0.954
Disease Severity				
Sofa	6.2 ± 3.5	5.2 ± 2.9	8.6 ± 3.7	< 0.001
APACHEII	21.2 ± 5.9	19.9 ± 4.8	24.4 ± 7.0	< 0.001
Septic shock	63 (26.7)	25 (15)	38 (55.1)	< 0.001
No_organ_failure				< 0.001
0	78 (33.1)	67 (40.1)	11 (15.9)	
1	80 (33.9)	63 (37.7)	17 (24.6)	
2	46 (19.5)	28 (16.8)	18 (26.1)	
3	32 (13.6)	9 (5.4)	23 (33.3)	
Laboratory Tests				
Hemoglobin	112.7 ± 29.5	113.7 ± 27.3	110.3 ± 34.3	0.427
Platelet	208.6 ± 113.9	220.7 ± 117.5	178.3 ± 99.1	0.01
PT	16.1 ± 7.4	14.5 ± 5.7	20.1 ± 9.4	< 0.001
APTT	33.6 ± 12.4	31.3 ± 10.0	39.3 ± 15.6	< 0.001
Fibrinogen	4.5 ± 1.8	4.5 ± 1.8	4.3 ± 1.8	0.445
D-Dimer	4.7 ± 5.4	3.7 ± 4.9	7.2 ± 5.8	< 0.001
ALT*	24.8 (15.7, 53.4)	22.1 (14.5, 37.4)	35.8 (19.5, 79.5)	< 0.001
AST*	28.2 (17.7, 54.3)	24.4 (16.7, 43.1)	45.4 (26.2, 71.5)	< 0.001
Albumin	32.2 ± 7.2	33.3 ± 7.1	29.5 ± 6.9	< 0.001
Bun*	8.0 (5.4, 14.1)	6.7 (4.8, 10.7)	15.4 (8.6, 25.0)	< 0.001
Scr*	66.0 (48.0, 96.5)	67.0 (54.1, 90.0)	54.5 (6.2, 125.2)	0.241
BNP*	192.9 (64.0, 1357.1)	111.4 (43.3, 623.6)	1058.4 (142.6, 2799.6)	< 0.001
Inflammatory and Immune test				
White blood cells	10.8 ± 6.2	10.7 ± 5.7	10.9 ± 7.5	0.825
CRP*	51.2 (10.8, 138.6)	48.9 (12.2, 115.9)	64.6 (10.1, 172.6)	0.365
T cells*	478.5 (255.5, 815.8)	599.0 (371.0, 1043.0)	254.0 (139.0, 450.0)	< 0.001
NK cells*	112.5 (63.2, 179.8)	123.0 (69.0, 212.0)	92.0 (42.0, 156.0)	0.005
B cells I6*	116.0 (52.0, 220.5)	130.0 (68.0, 250.0)	80.0 (32.0, 138.0)	< 0.001
IL-6*	96.3 (33.3, 411.0)	75.4 (22.1, 163.2)	469.9 (98.3, 1993.6)	< 0.001
IL-8*	319.0 (143.5, 901.1)	220.6 (114.8, 644.2)	664.3 (284.0, 1615.7)	< 0.001
IL-10*	8.3 (4.8, 47.4)	6.4 (3.9, 14.4)	62.0 (14.7, 225.1)	< 0.001

**Notes:** \*Variables with non-normal distribution (ALT, AST, BUN, Scr, BNP, CRP, T cells, NK cells, B cells, IL-6, IL-8, IL-10) are presented as median (interquartile range, IQR). All other continuous variables are presented as mean ± standard deviation (SD). Categorical variables are presented as n(%).

Prognostic Analysis

In a univariate Cox regression analysis of 236 sepsis patients, multiple factors were found to significantly influence 28-day mortality, as detailed in Table 2. Each additional year of age increased mortality risk by 2% (HR = 1.02, p = 0.014). Coronary heart disease and heart failure increased death risks by 70% (HR = 1.70, p = 0.033) and 149% (HR = 2.49, p = 0.003), respectively. Chronic kidney disease was associated with a 142% higher risk (HR = 2.42, p = 0.004). Disease severity significantly impacted mortality, with each increase in SOFA and APACHE II scores raising risk by 22% (HR = 1.22, p < 0.001) and 8% (HR = 1.08, p < 0.001), respectively. Septic shock was linked to a 185% increased risk of death



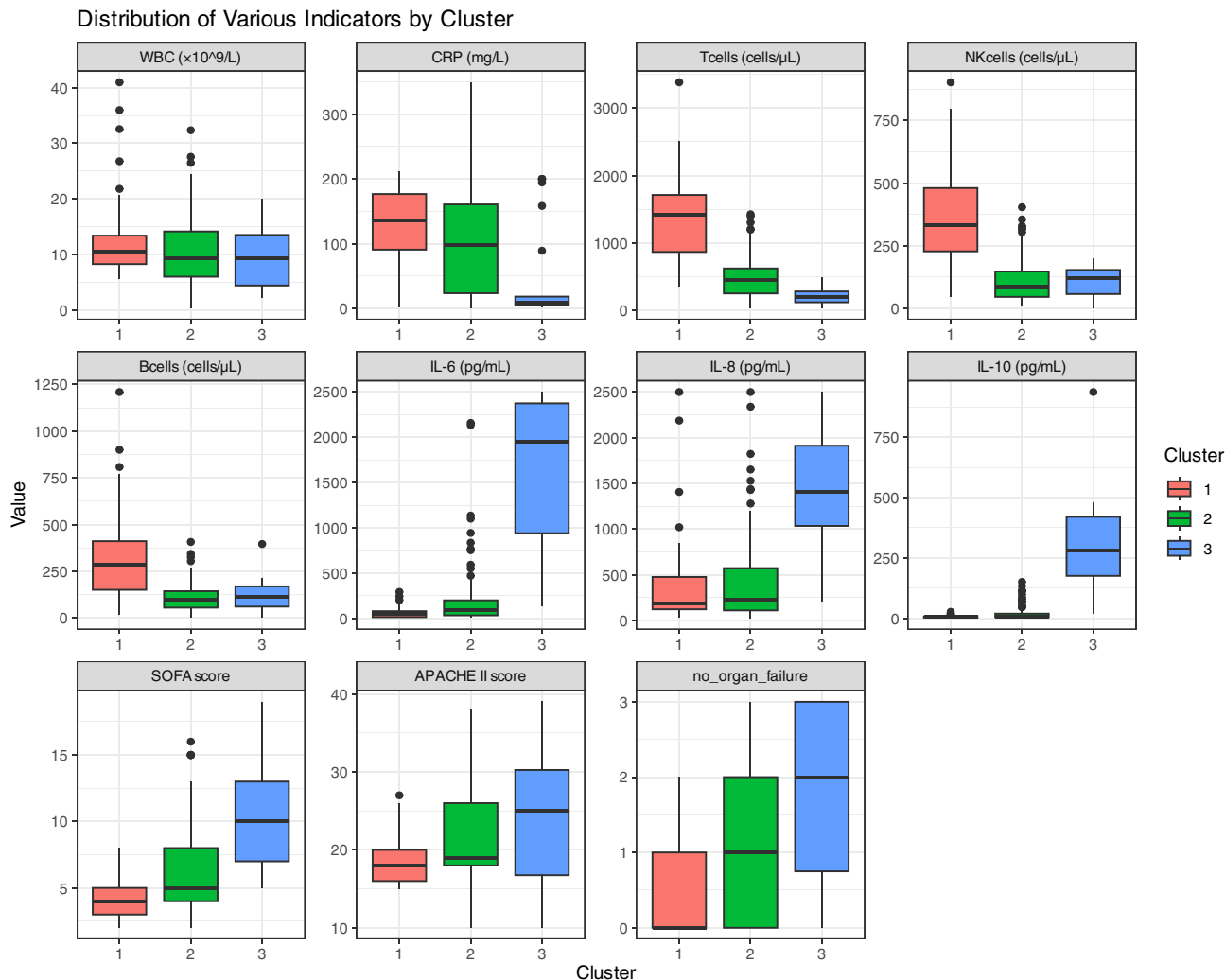
**Figure 1** Clustering Analysis of Sepsis Immunotypes.

(HR = 2.85,  $p < 0.001$ ), and patients in Cluster 3 had a 21.65-fold higher risk compared to Cluster 1 (HR = 21.65,  $p < 0.001$ ). Key laboratory indicators also correlated with mortality: lower platelet counts, prolonged prothrombin time (PT) and activated partial thromboplastin time (APTT), and elevated D-dimer, AST, blood urea nitrogen (BUN), and brain natriuretic peptide (BNP) levels significantly increased mortality risks, with each variable's increase linked to a corresponding rise in death risk.

In multivariate Cox regression analysis, three models were constructed to evaluate the association between clusters and 28-day mortality, as shown in Table 3. Without adjusting for any covariates, patients in Cluster 2 had a 2.74-fold increased risk of death, although this result approached statistical significance (HR = 2.74, 95% CI: 0.98–7.66,  $p = 0.055$ ). Patients in Cluster 3 had a significantly increased risk of death by 21.65-fold (HR = 21.65, 95% CI: 7.46–62.87,  $p < 0.001$ ). After adjusting for demographic information including age, sex, and underlying diseases, patients in Cluster 2 had a 2.16-fold increased risk of death, although this result did not reach statistical significance (HR = 2.16, 95% CI: 0.76–6.19,  $p = 0.151$ ). Patients in Cluster 3 had a significantly increased risk of death by 20.4-fold (HR = 20.4, 95% CI: 6.54–63.6,  $p < 0.001$ ). After further adjusting for variables that were significant in the univariate Cox regression analysis, patients in Cluster 2 had a 1.68-fold increased risk of death, although this result did not reach statistical significance (HR = 1.68, 95% CI: 0.37–7.71,  $p = 0.502$ ). Patients in Cluster 3 had a significantly increased risk of death by 19.5-fold (HR = 19.5, 95% CI: 3.07–123.72,  $p = 0.002$ ). Although patients in Cluster 2 showed an increased risk of death in all models, it only approached statistical significance in Model 1. Patients in Cluster 3 consistently showed a significantly increased risk of death across all models, indicating that patients in Cluster 3 had a significantly higher risk of death compared to other groups.

## Kaplan-Meier Survival Curves

The Kaplan-Meier survival curves demonstrated the survival patterns of sepsis patients with different immune response subtypes. As shown in Figure 3. Patients with the high immune activation subtype had the highest survival rate, with



**Figure 2** Distribution of Various Clinical Indicators by Cluster in Sepsis Patients.

a survival probability of over 80% at 28 days and a gradual decline in the survival curve. Patients with the moderate immune activation subtype had an intermediate survival rate, with a survival probability of approximately 50% at 28 days and a decline rate between that of Cluster 1 and Cluster 3. Patients with the high inflammation and immunosuppression subtype had the lowest survival rate, with a survival probability of less than 20% at 28 days and a sharp decline in the survival curve, particularly within the first 10 days. The Log rank test indicated highly significant differences in survival among the three groups ( $p < 0.0001$ ). This suggests that sepsis patients with different immune response subtypes exhibit significant differences in survival rates, further emphasizing the importance of subtype classification based on immune and inflammatory characteristics in prognostic assessment of sepsis patients.

## Discussion

Currently, sepsis is defined as life-threatening organ dysfunction caused by a dysregulated or dysfunctional host immune response to infection.<sup>11,12</sup> However, the nature and mechanisms of immune dysregulation in sepsis remain unclear.<sup>13</sup> This study utilized K-means clustering analysis, an unsupervised learning technique, to identify three immune subtypes in sepsis patients: high immune activation, moderate immune activation, and high inflammation and immunosuppression. The results showed that patients in the high inflammation and immunosuppression subtype had a significantly higher 28-day mortality risk, while those in the high immune activation subtype had the highest survival rate. These findings

**Table 2** Univariate Cox Regression Analysis of Risk Factors for 28-Day Mortality

Variables	HR(95% CI)	p
Age	1.02 (1,1.04)	0.014
Male	0.86 (0.52,1.42)	0.561
Hypertension	1.33 (0.82,2.17)	0.249
Diabetes Mellitus	1.04 (0.64,1.68)	0.875
Coronary Heart Disease	1.7 (1.04,2.78)	0.033
Heart Failure	2.49 (1.35,4.57)	0.003
Chronic Kidney Disease	2.42 (1.32,4.43)	0.004
COPD	1.59 (0.86,2.97)	0.142
Sofa	1.22 (1.15,1.3)	< 0.001
Apache ii	1.08 (1.03,1.12)	< 0.001
Septic shock	2.85 (1.77,4.6)	< 0.001
Cluster		
1		
2	2.74 (0.98,7.66)	0.055
3	21.65 (7.46,62.87)	< 0.001
Hemoglobin	1.0031 (0.9936,1.0127)	0.529
Platelet	0.9976 (0.9953,0.9998)	0.035
PT	1.09 (1.06,1.11)	< 0.001
APTT	1.02 (1.01,1.03)	< 0.001
Fibrinogen	0.95 (0.82,1.1)	0.49
D-Dimer	1.06 (1.02,1.1)	0.001
ALT	1.001 (0.9996,1.0024)	0.145
AST	1.0011 (1.0002,1.002)	0.012
Albumin	0.96 (0.92,1)	0.059
Bun	1.06 (1.04,1.08)	< 0.001
Scr	0.9986 (0.9955,1.0018)	0.394
BNP	1.0004 (1.0003,1.0006)	< 0.001

**Table 3** Multivariate Cox Regression Analysis of Risk Factors for 28-Day Mortality

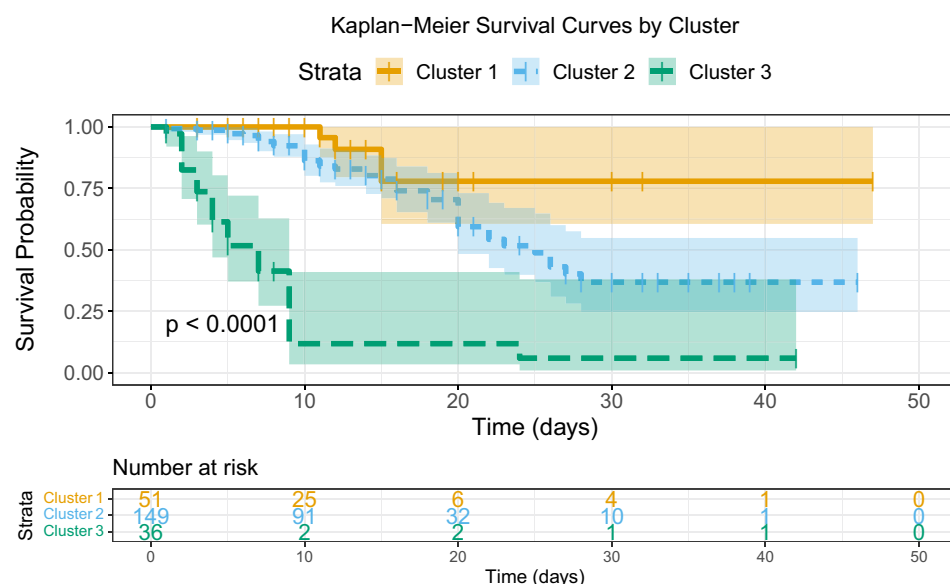
Variable	Model 1	p	Model 2	p	Model 3	p
Cluster 1	Ref		Ref		Ref	
Cluster 2	2.74 (0.98~7.66)	0.055	2.16 (0.76~6.19)	0.151	1.68 (0.37~7.71)	0.502
Cluster 3	21.65 (7.46~62.87)	<0.001	20.4 (6.54~63.6)	<0.001	19.5 (3.07~123.72)	0.002
p for trend	6.44 (3.99~10.38)	<0.001	6.94 (3.96~12.14)	<0.001	7.6 (2.89~19.99)	<0.001

**Notes:** Model 1: Without adjusting for any covariates. Model 2: After adjusting for demographic information including age, sex, and underlying diseases. Model 3: After further adjusting for variables that were significant in the univariate Cox regression analysis.

provide new scientific evidence for personalized treatment of sepsis, potentially optimizing therapeutic strategies and improving patient outcomes.

Several existing studies have identified different sepsis endotypes based on whole blood leukocyte gene expression profiles in sepsis patients. In patients with community-acquired pneumonia-induced sepsis, two Sepsis Response Signatures were identified: SRS1 and SRS2. SRS1 was associated with higher mortality and exhibited an immunosuppressive phenotype, with gene expression profiles showing endotoxin tolerance, HLA class II downregulation, and T cell exhaustion.<sup>14</sup> Furthermore, SRS1 and SRS2 endotypes were validated in a retrospective analysis of a clinical trial on corticosteroid treatment in septic shock patients. The study found that corticosteroid treatment appeared harmful in SRS2





**Figure 3** Kaplan-Meier Survival Curves by Sepsis Immunotype Clusters.

endotype patients while showing no significant treatment effect in SRS1 patients, suggesting the potential importance of SRS1 and SRS2 stratification in treatment decisions.<sup>15</sup> Another team reported four transcriptomic endotypes in sepsis patients, named Mars1 to Mars4.<sup>16</sup> Mars1 was associated with increased mortality across different cohorts, with gene expression profiles indicating suppression of both innate and adaptive immunity, while Mars3 had relatively lower mortality risk, with transcriptomic features indicating upregulation of adaptive immunity and enhanced T cell function.<sup>16</sup> Comparative analysis showed that Mars3 overlapped with the previously described SRS2 endotype.<sup>14,16</sup> Another study identified three subgroups based on blood transcriptomics, named “inflammatory” (characterized by innate immune activation and higher mortality), “adaptive” (adaptive immune activation, lower mortality), and “coagulopathic” (platelet degranulation and coagulation dysfunction, higher mortality and older age).<sup>17</sup> A recent study identified five endotypes in early sepsis patients, named “neutrophil suppressive” (associated with neutrophil activation and immunosuppression), “inflammatory” (enhanced pro-inflammatory response), “innate host defense” (interleukin signaling), “interferon” (increased IFN- $\alpha$ ,  $\beta$ ,  $\gamma$ ), and “adaptive” (activation of multiple pathways including enhanced adaptive immunity).<sup>18</sup> Our study identified three immune subtypes through cluster analysis: high immune activation, moderate immune activation, and high inflammation and immunosuppression. Similar to the existing SRS and Mars endotypes, patients in the high inflammation and immunosuppression subtype showed a higher risk of mortality, consistent with the characteristics of SRS1 and Mars1 endotypes. This finding further supports the importance of the immunosuppressive phenotype in sepsis patients associated with poor outcomes. Our study also found that patients in the high immune activation subtype had the highest survival rate, which is consistent with the low mortality risk of Mars3 and SRS2 endotypes. While previous studies primarily focused on genomic profiling, our study uniquely integrates clinically accessible immune parameters and inflammatory markers, offering practical advantages for routine clinical assessment. Furthermore, our clustering analysis approach provides comprehensive immune status evaluation by integrating multiple parameters (CRP, WBC, T cells, NK cells, B cells, IL-6, IL-8, and IL-10), which offers advantages over single-parameter approaches. Single predictors such as IL-6, BUN, or age alone may reflect only limited aspects of the complex sepsis pathophysiology, whereas our integrated approach captures the multifaceted nature of immune dysregulation in sepsis. This comprehensive evaluation enables more nuanced patient stratification and targeted therapeutic strategies. For example, patients in different clusters may benefit from distinct immunomodulatory approaches, facilitating personalized treatment decisions in clinical practice. The identification of three distinct immune subtypes through multiple parameter integration provides a more robust framework for clinical decision-making compared to traditional single-parameter assessments.



The high immune activation subtype exhibited high levels of CRP and WBC, as well as high levels of T cells, NK cells, and B cells, indicating a strong immune response to infection in these patients. This high immune response may be due to a high pathogen load or differences in host genetics, enabling rapid mobilization of immune cells to control infection. However, excessive immune activation may also lead to tissue damage and organ dysfunction. The moderate immune activation subtype showed moderate levels of immune and inflammatory markers, possibly representing a balanced immune response state. These patients' immune systems can effectively respond to infection while avoiding excessive inflammatory responses, thereby reducing the risk of immune-related damage. Patients in the high inflammation and immunosuppression subtype exhibited very high levels of IL-6, IL-8, and IL-10, but low levels of T cells, NK cells, and B cells. This suggests that these patients may have experienced immunosuppression and cellular function exhaustion.<sup>19</sup> IL-10 is an anti-inflammatory cytokine known to play a role in regulating inflammatory responses in sepsis, and its high levels may represent an inhibitory feedback to persistent inflammatory responses.<sup>20</sup> However, this feedback mechanism may be insufficient to counteract excessive inflammatory responses, leading to persistent tissue damage and higher mortality risk. The findings of this study have important clinical implications. Firstly, the significant differences in survival rates among different immune subtypes suggest that clinicians should develop personalized treatment plans based on patients' immune characteristics. For patients in the high inflammation and immunosuppression subtype, more aggressive anti-inflammatory and immune support treatments may be needed, while for patients in the high immune activation subtype, monitoring and prevention of excessive immune responses may be necessary. Additionally, the clustering method and results provided by this study can also be used to identify immune subtypes in other severe infectious diseases, providing a reference for broader clinical applications.

Despite the important findings of this study, there are some limitations that warrant discussion. Firstly, this is a single-center study with a sample size of 236 patients, which may affect the external validity of the results. The immune subtypes identified might be influenced by the characteristics of our specific patient population, and results could differ in other healthcare settings or populations. Due to these limitations, our findings should be considered preliminary and hypothesis-generating. Secondly, this study did not cover all possible factors that may influence sepsis prognosis, such as genetic background, differences in specific infectious pathogens, and variations in initial therapeutic interventions. Additionally, the dynamic changes of immune parameters during the disease course were not captured in our analysis. Future studies should expand the sample size, validate these findings in multi-center cohorts with diverse patient populations, and incorporate more potential influencing factors to improve the generalizability and accuracy of the results. Furthermore, longitudinal studies tracking the evolution of these immune subtypes during the course of sepsis would provide valuable insights into their stability and clinical utility.

## Conclusion

This study identified three distinct immunotypes of sepsis through cluster analysis and revealed their significant associations with clinical outcomes. These findings provide new scientific evidence for personalized treatment of sepsis, with the potential to optimize therapeutic strategies and improve patient prognosis. Future research should further validate these findings and explore additional potential influencing factors to advance the development of personalized treatment for sepsis.

## Disclosure

Jian Zhao, Rushun Dai, Yi Zhao and Jiaping Tan contributed equally to this work as co-first authors and should be considered joint first authors. The authors report no conflicts of interest in this work.

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