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The Predictive Value of Absolute Lymphocyte Count and T Cell Subpopulations for Sepsis Prognosis

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Background: Sepsis causes substantial morbidity and mortality and constitutes a major public health problem. In patients with sepsis, immunosuppression is associated with poor prognosis, and immune monitoring during the early stages has prognostic value. This study aims to explore immunologic parameters associated with sepsis prognosis, potentially identifying patients who may benefit from immunotherapy, improving intensive care survival.

Methods: A total of 65 patients with sepsis from the Department of Emergency Medicine were divided based on survival at 28 days (47 in the survival group, 18 in the non-survival group). Peripheral blood was collected to measure absolute lymphocyte count and T lymphocyte subpopulations, including the percentage and absolute count of total T cells, $CD4^+$ T, $CD8^+$ T, and NK cells, and the percentages of naïve $CD4^+$ T, central memory $CD4^+$ T, effector $CD4^+$ T, effector memory $CD4^+$ T, naïve $CD8^+$ T, central memory $CD8^+$ T, effector $CD8^+$ T, effector memory $CD8^+$ T, $CD4^+$HLA-DR^+ T, and $CD8^+$HLA-DR^+ T cells, and Tregs. The differences in these parameters between the two groups were compared and a regression model was constructed to identify possible risk factors for death in patients with sepsis.

Results: The absolute lymphocyte count, absolute T cell count ($CD3^+$, $CD4^+$, and $CD8^+$) and naïve $CD4^+$ T cell percentage were significantly lower in the non-survival group. Conversely, Tregs were higher in patients who did not survive sepsis. In regression analysis, the absolute lymphocyte count and naïve $CD4^+$ T cell percentage remained statistically significant. The receiver operating characteristic curve showed that a model based on the absolute lymphocyte count (435 cells/μL) and naïve $CD4^+$ T cell percentage (20.25%) performed best in predicting sepsis prognosis.

Conclusion: Monitoring of absolute lymphocyte count and analysis of T cell subtypes in the early phase of sepsis is predictive of outcome and may help identify those patients who would benefit from immunotherapy, improving survival.

Keywords: sepsis, absolute lymphocyte count, T cell subpopulations, immune function

Introduction

The 2016 consensus guidelines on sepsis define sepsis as life-threatening organ dysfunction caused by dysregulated host response to infection.[1] Sepsis is thought to result from an imbalanced immune response in which pathogens escape immune monitoring and proliferate. This imbalance results in repeated immune stimulation and host damage, with the body unable to restore balance.[2] Over the past few decades, researchers have made significant advances in the early diagnosis and treatment of sepsis. Commonly used clinical assessment tools include the APACHE II score, SOFA score, as well as biomarkers such as C-reactive protein (CRP) and procalcitonin (PCT).[1,3] The APACHE II score has the

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advantage of integrating multiple physiological parameters, age, and chronic health conditions; however, its complexity necessitates extensive clinical data, and it may not be sufficiently sensitive to individual patient variations.[1] This is particularly relevant in emergency settings, where rapid and accurate assessment of a patient's condition, as well as preliminary prognostic judgments, are crucial for clinical decision-making. The SOFA score is widely used in intensive care units (ICUs) and focuses more on assessing organ function. However, it may not accurately predict outcomes in the early stages of sepsis, because organ failure can develop later than the initial changes associated with sepsis.[4] Clinically, lactate levels, CRP, and PCT are frequently monitored. Elevated lactate levels are typically correlated with tissue hypoxia and the severity of sepsis, making it a sensitive prognostic marker. However, lactate elevation can be triggered by various factors and is not specific to sepsis; it may also be influenced by individual patient differences. CRP is another commonly used clinical marker for the presence of sepsis and for monitoring its severity, but its elevation can result from various inflammatory conditions. Similarly, PCT is released in large quantities during exacerbation of infection and is often used clinically to diagnose infections and assess their severity; however, its specificity is low in many non-infectious conditions and in cases of viral and bacterial infections.[3] While these traditional prognostic factors hold some value in assessing sepsis patients, they also have limitations, such as insufficient sensitivity to early disease changes, inherent complexity, or lack of specificity. Therefore, the identification of new effective biomarkers, such as lymphocyte counts and T and B lymphocyte subpopulations, is of significant importance for improving the accuracy and timeliness of prognostic predictions.

In recent years, the focus in sepsis research has shifted from the cause of infection to the host response. Of all host reactions evaluated, immunosuppression has been found to be associated with poor prognosis in sepsis, leading to poor control of the primary infection and development of lethal secondary infection.[5,6] Patients with sepsis and immunosuppression (including congenital immunodeficiency and those who have undergone hematopoietic cell transplantation) or immune paralysis are considered high-risk.[4,7–9] Immune response in humans consists of the innate immune system, which acts early and is universal against all infections, and the adaptive immune system, which generates specific immune response to the infection. Sepsis alters the production, longevity, and function of immune effector cells, severely impacting both the innate and adaptive immune system, resulting in immune suppression. The specific mechanisms by which this alteration leads to immunosuppression are complex, involving multiple cell phenotypes. These mechanisms includes increased immune cell apoptosis, T cell exhaustion, epigenetic changes causing immune cell reprogramming, and reduced expression of cell surface activation markers.[2,10] Immunosuppression not only promotes sepsis development but also is associated with poor prognosis and development of long-term sequelae after sepsis.[11] Recent developments in immunoassay technology have significantly improved our ability to understand the impact of lymphocyte subsets in sepsis.[12–14]

Lymphocyte exhaustion is a characteristic change of immune cells in sepsis. Multiple studies have demonstrated the changes of host immune response in sepsis, and indicators such as lymphocyte count and T and B lymphocyte subsets can be used as indicators to evaluate host immune status.[11,15–17] T lymphocytes play an especially crucial role in the adaptive immune response to sepsis.[18] With recent developments in immunology, lymphocytes can be more finely grouped. T lymphocytes are divided into naïve, effector, central memory, and effector memory T cells. $CD4^+$ T helper cells can be further divided into Th1-like, Th2-like, Th17-like, Th9-like, and Tregs according to their cell surface markers and the different cytokines secreted. Refining the analysis of circulating T-lymphocyte subpopulations can facilitate monitoring disease onset and progression, as well as aiding in determining optimal treatment strategies.[19] In this study, the difference of T-lymphocyte profiles in peripheral blood between survivors and non-survivors of sepsis is compared to identify parameters correlated with prognosis.

Materials and Methods

Participants

A total of 71 patients with sepsis treated in the Department of Emergency Medicine of Peking University First Hospital from August 2021 to December 2022 were enrolled in this retrospective study. For the purposes of this study, sepsis has been defined as a score≥2 points in the sepsis-related organ failure assessment (SOFA), based on proven or suspected infection.[20] The exclusion criteria were: pregnant or breastfeeding, proliferative hematologic disorders, immune system

disease and immune dysfunction, or receiving immunosuppressants or corticosteroids. All patients received appropriate treatment in the Peking University First Hospital emergency department. The participants were divided into two groups based on survival 28 days after admission.

This study has been approved by the Ethics Committee of Peking University First Hospital and has been carried out in accordance with *The Code of Ethics of the World Medical Association (Declaration of Helsinki)* for experiments involving humans and its later amendments. The ethical No. is 2023–011.

Data Collection

Clinical data Collection

Pertinent clinical information including demographic data (age, gender), underlying condition or illness, a SOFA score, treatment given, and the clinical outcome (28-day survivor or non-survivor) were obtained from the clinical electronic medical record system.

Laboratory Analysis

For patients with sepsis, blood specimens were typically collected shortly after admission, and always on day 1 or 2 after sepsis was identified. We collected 2 mL of venous blood in EDTA tubes for a complete blood count (CBC) and T-lymphocyte subset quantification and phenotype analysis. The CBC was obtained using a hematology analyzer (Beckman Coulter DxH800). Parameters included white blood cells (WBC count), neutrophil percentage (NEU%), absolute neutrophil count (NEU#), lymphocyte percentage (LYM%), and absolute lymphocyte count (LYM#).

T-Lymphocyte Subset Counting and Subpopulation Phenotype Analysis

To quantify T-lymphocyte subsets and perform subpopulation phenotype analysis, we used flow cytometric analysis (BD FACS Canto II flow cytometer). The following parameters were assessed: 1) T cell populations: The percentage and absolute count of $CD3^+$ T cells, $CD4^+$ T cells, $CD8^+$ T cells and $CD3^-CD56^+$ NK cells were counted by flow cytometry using TruCOUNT tubes. The procedure, in brief, was: 50μL of whole blood was labeled with 4-color fluorescein-labeled monoclonal antibodies (CD3FITC-CD8PE-CD45PerCP-CD4APC and CD3FITC-CD16+56PE-CD45PerCP-CD19APC, BD Biosciences) for 15 min at room temperature in the dark. The red blood cells were then lysed by incubation with lysis buffer (BD Biosciences) for 10 min. The data were analyzed using FACSCANTO (BD Biosciences) software. 2) $CD4^+$ T cell subpopulations and $CD8^+$ T cell subpopulations: Using sequential analysis gates, $CD3^+$ T cells were identified, then T helper cells ($CD3^+CD4^+$) and cytotoxic T cells ($CD3^+CD8^+$) were further delineated. Subsets of $CD4^+$ T and $CD8^+$ T cells were categorized based on marker expression into naïve, central memory, effector, and effector memory T cells.[21] Activated T cells were identified based on expression of HLA-DR markers. 3) Tregs: T helper cells with the staining characteristics of $CD25^+CD127^{low}CCR4^+$ were categorized as Tregs.[21] The marker expression used to categorize $CD4^+$ T and $CD8^+$ T cell subpopulations is listed in Table 1. The percentages of cells with these phenotypes were calculated using FACS Diva (BD Biosciences) software. The representative flow cytometry charts for the phenotype analysis gating strategies are shown in Figure 1.

Statistical Analysis

Continuous data were expressed as means ± SD for normally distributed variables, or as medians with interquartile ranges (25th-75th percentiles) for non-normally distributed variables. Categorical data were reported as percentages. For comparison of two groups of continuous variables, we used Student's *t*-test (for normally distributed variables) or the Mann–Whitney *U*-test (for non-normally distributed variables). The $\chi 2$ test or Fisher's exact test was used for the comparison of categorical variables. All tests were two-tailed. Findings were considered significant when $p < 0.05$ unless otherwise specified.

To examine independent variables related to death, logistic regression analysis was conducted. Given the population enrolled, we included variables with $p<0.05$ in the multivariate logistic regression analysis to construct the regression equation (prediction model) and evaluate risk factors associated with death of patients with sepsis. The receiver operating characteristic (ROC) curve analysis was used to evaluate the performance of predictive models for the prognosis of sepsis.

Table 1 The Marker Expression of $CD4^+$ T and $CD8^+$ T Cells Subpopulations

	Category Name	Marker Expression
Subsets of $CD4^+$ T cells	**Naïve $CD4^+$ T cells**	$CCR7^+CD45RA^+$ $CD45RO^-$ $CD4^+$
	Central memory $CD4^+$ T cells	$CCR7^+CD45RA^-CD45RO^+$ $CD4^+$
	Effector $CD4^+$ T cells	$CCR7^-CD45RA^+CD45RO^-$ $CD4^+$
	Effector memory $CD4^+$ T cells	$CCR7^-CD45RA^-CD45RO^+$ $CD4^+$
	$CD4^+$HLA-DR^+ T cells	HLA-DR^+ $CD4^+$
	Tregs	$CD25^+CD127^{low}CCR4^+$ $CD4^+$
Subsets of $CD8^+$ T cells	**Naïve $CD8^+$ T cells**	$CCR7^+CD45RA^+CD45RO^-$ $CD8^+$
	Central memory $CD8^+$ T cells	$CCR7^+CD45RA^-CD45RO^+$ $CD8^+$
	Effector $CD8^+$ T cells	$CCR7^-CD45RA^+CD45RO^-$ $CD8^+$
	Effector memory $CD8^+$ T cells	$CCR7^-CD45RA^-CD45RO^+$ $CD8^+$
	$CD8^+$HLA-DR^+ T cells	HLA-DR^+ $CD8^+$

Data analysis was performed using IBM SPSS Statistics software (version 26, SPSS, Inc., Chicago, IL, USA) and GraphPad Prism (version 9.4.1, GraphPad Software, San Diego, CA, USA). In order to reduce bias in obtaining and analyzing the data, we separated sources of information and arranged for data collection by people who were unaware of

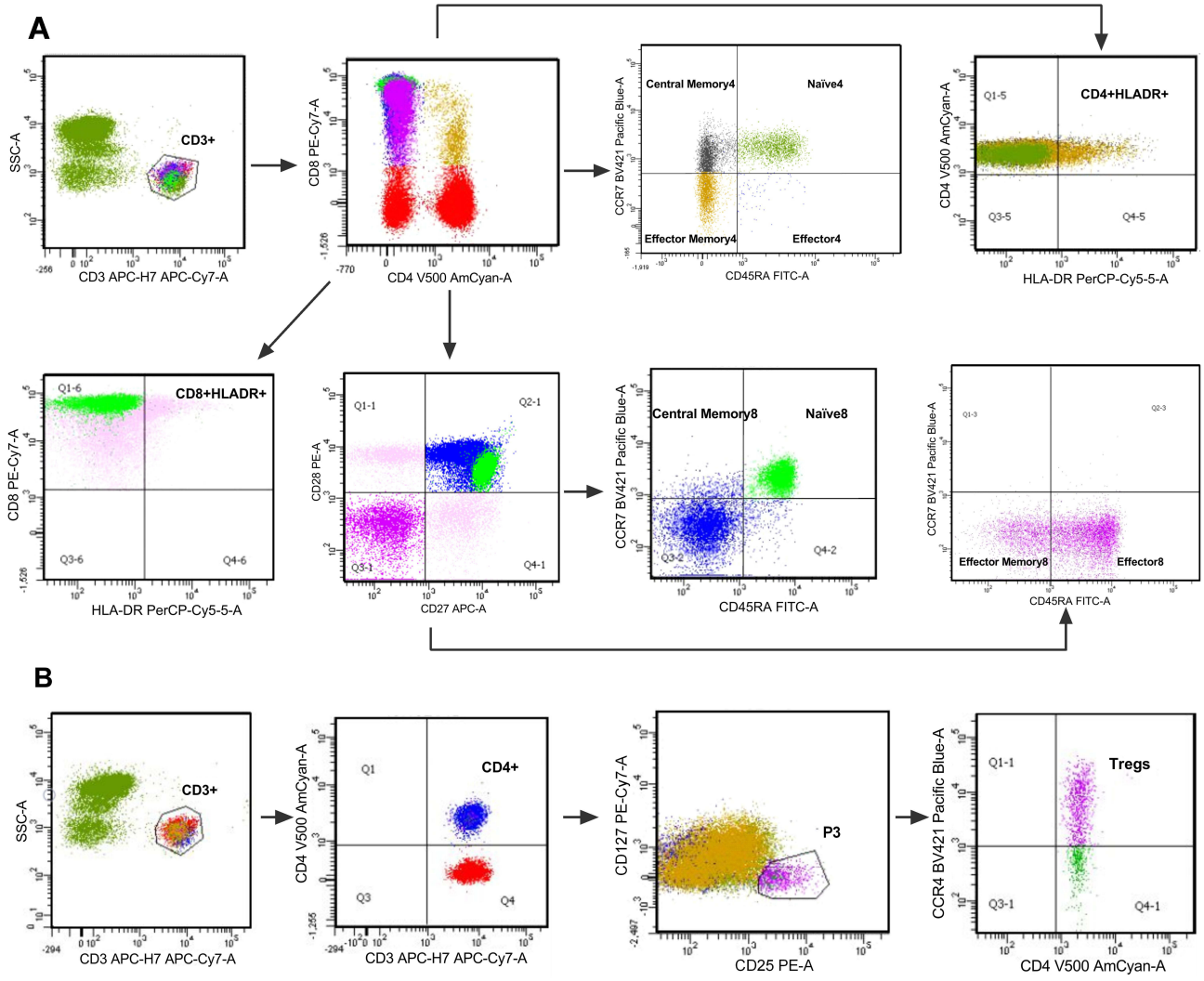

Figure 1 Representative flow cytometry gating strategies for phenotype analysis of T cell subsets. (**A**) The gating strategies for phenotype analysis of $CD4^+$T cell and $CD8^+$T cell subpopulations; (**B**) The gating strategy for Tregs.

the purpose of the study and information about patient subgroups. Furthermore, we have carried out an independent analysis, where the researcher responsible for the data analysis was unaware of the study context, subgroups of patients, or the specific hypotheses and aims of the study. This blinding served to minimize subjective bias in the analysis process.

Results

Patient Demographics and Clinical Characteristics

A total of 71 patients were recruited in our study (47 males, 24 females; aged 33 to 92 years, median 73 years; IQR 65–83 years). Six patients were excluded based on the exclusion criteria above. Among the 65 patients remaining, 72.31% were survivors (47/65) and 27.69% were non-survivors (18/65). Non-survivors had higher initial illness severity based on SOFA scores obtained within 24 h of admission compared with survivors. The demographic characteristics, SOFA scores, and clinical characteristics of the patients (survivors and non-survivors) are summarized in Table 2.

Comparison of T-Lymphocyte Subsets

Between the two groups, the absolute lymphocyte count, and the percentage and absolute count of T cells ($CD3^+$ T cells, $CD4^+$ T cells, $CD8^+$ T cells) and NK cells ($CD3^-CD56^+$) were lower in the non-survival group. The differences were statistically significant for absolute lymphocyte count (p=0.008), $CD3^+$ T cell absolute count (p=0.015), $CD4^+$ T cell count (p=0.019) and $CD8^+$ T cell count (p=0.043). These results are summarized in Table 3 and Figure 2.

T-Lymphocyte Subpopulations Analysis

We initially compared the percentage of T-lymphocyte subpopulations in patients with sepsis to the reference range of healthy adults established in our laboratory (Table 4). The analysis revealed that the variation range of T cell subpopulations in patients with sepsis was significantly wider than the reference range of healthy controls (Figure 3). This observation suggested that immune function, especially adaptive immunity, is markedly altered in patients with sepsis. The median and most data values of effector $CD4^+$ T cells, naïve $CD8^+$ T cells, and central memory $CD8^+$ T cells were lower than the lower limit of the reference interval in both the non-survival and survival groups (Figure 3). Conversely, the values for $CD4^+$HLA-DR^+ T cells and $CD8^+$HLA-DR^+ T cells were higher than the upper limit of the reference interval in both groups, suggesting that activated T cells increase in sepsis. We also calculated the proportion of values above (or below) the reference range in the two groups for each parameter assessed. The percentages of each group that were below the lower limit of the reference interval and above the upper limit of the reference interval are shown in Table 4.

We then compared the difference of T-lymphocyte subpopulations between survivors and non-survivors of sepsis, including the $CD4^+$ and $CD8^+$ T cell subpopulations. The percentage of naïve $CD4^+$ T cells, naïve $CD8^+$ T cells, and effector $CD8^+$ T cells were lower in non-survivors, though only the naïve $CD4^+$ T cells were statistically significant

Table 2 The Clinical and Demographic Characteristics of the Study Participants

	Survivors (n = 47)	Non-survivors (n = 18)	*p*-value
Age, median (IQR)	72 (62–82)	78 (70–86)	p=0.076
Males, n (%)	26 (55.32%)	16 (88.89%)	p=0.025
SOFA score	4 (2–6)	7.5 (5–13)	p=0.001
Underlying illness,n (%)			
Cancer	3 (6.4%)	5 (27.8%)	p=0.054
Coronary artery disease	16 (34.0%)	9 (50.0%)	p=0.237
Diabetes mellitus	19 (40.4%)	2 (11.1%)	p=0.049
Hypertension	25 (53.2%)	3 (16.7%)	p=0.017
Chronic pulmonary disease	29 (63.8%)	16 (83.3%)	p=0.068
Chronic kidney disease	9 (19.1%)	5 (27.8%)	p=0.449

Table 3 The Laboratory Observation Index of the Study Participants

Variable	Survivors (n = 47)	Non-survivors (n = 18)	*p*-value
WBC count (× 10^9/L)	9.83 (7.31–12.15)	10.05 (5.81–12.46)	*p*=0.977
Neutrophil AC (/μL)	7839.20 (5431.33–10,802.82)	8697.84 (4690.96–11,374.69)	*p*=0.692
Lymphocyte AC (/μL)	830 (560–1140)	505 (222.5–900)	*p*=0.008
$CD3^+$ T cells (%)	68.02 ±12.42	63.51 ±12.69	*p*=0.197
$CD3^+$ T cells AC (/μL)	563.14 (385.13–789.42)	311.42 (77.83–586.81)	*p*=0.015
$CD4^+$ T cells (%)	40.12 ±11.76	38.26 ±14.17	*p*=0.593
$CD4^+$ T cells AC (/μL)	298.16 (224.91–478.00)	176.72 (54.44–306.83)	*p*=0.019
$CD8^+$ T cells (%)	25.00 (18.94–31.53)	21.42 (15.73–37.20)	*p*=0.567
$CD8^+$ T cells AC (/μL)	176.68 (121.57–355.57)	75.0 (34.01–292.72)	*p*=0.043
NK cells (%)	15.02 (9.03–21.43)	12.63 (11.30–27.90)	*p*=0.792
NK cells AC (/μL)	112.75 (57.87–202.01)	72.58 (37.51–181.13)	*p*=0.177
$CD4^+$/ $CD8^+$	1.57 (0.96–2.44)	2.12 (0.82–2.53)	*p*=0.769

Note: Data are presented as means ± SD, or medians (25th - 75th percentiles).
Abbreviation: AC, absolute count.

(*p*=0.012). Conversely, the percentages of central memory $CD4^+$ T cells, effector $CD4^+$ T cells, effector memory $CD4^+$ T cells, central memory $CD8^+$ T cells, effector memory $CD8^+$ T cells, $CD4^+$HLA-DR^+ T cells, $CD8^+$HLA-DR^+ T cells, and Tregs were higher among non-survivors, though only Tregs were significantly higher (*p*=0.017). These results are summarized in Table 4 and Figure 3.

Analysis of Risk Factors Associated with Mortality in Sepsis

To identify the risk factors impacting prognosis in sepsis, the statistically significant lymphocyte parameters identified above were further analyzed between the non-survival and survival groups, evaluating variables with $p<0.05$ by multivariable logistic regression analysis. The absolute lymphocyte count, absolute $CD3^+$ T cell count, absolute $CD4^+$ T cell

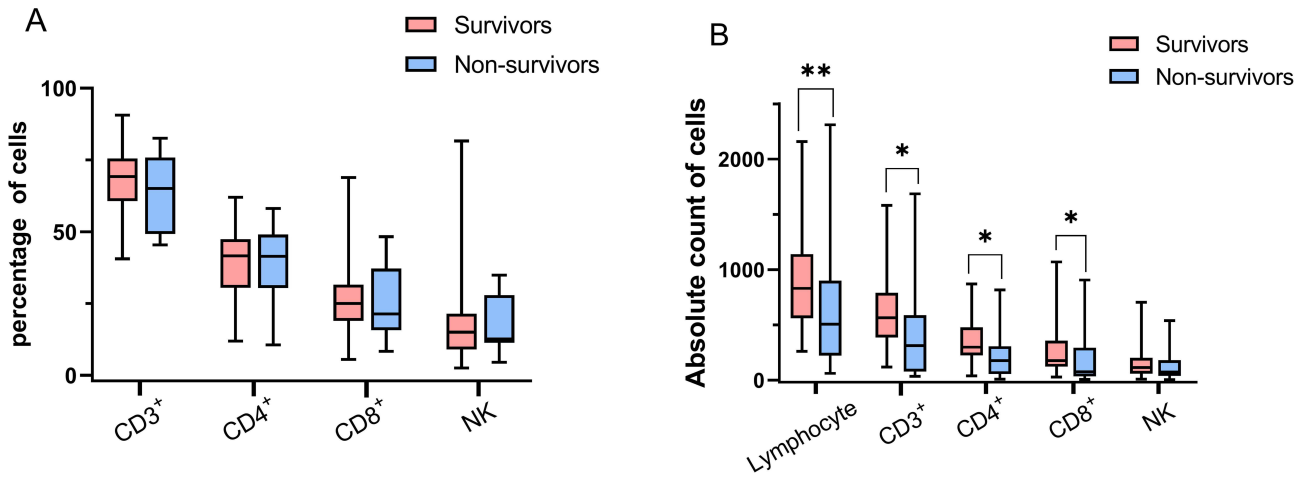

Figure 2 The percentage and absolute count of T-lymphocyte subsets in patients with sepsis having different outcomes. (**A**) Boxplots showing the percentage of $CD3^+$T cells, $CD4^+$T cells, $CD8^+$T cells and natural killer (NK) cells; (**B**) Boxplots showing the absolute count of lymphocytes, $CD3^+$T cells, $CD4^+$T cells, $CD8^+$T cells, and NK cells. (survivor n=47, non-survivor n=18) *P < 0.05, **P < 0.01 (Student's *t*-test or Mann–Whitney *U*-test). The boxplots depict the median, and 25th to 75th percentiles, and the whisker indicates the maximum and minimum values. NK cells: natural killer cells.

Table 4 The T-Lymphocyte Subpopulations of the Study Participants

Variable	The Difference Between the Two Groups			Comparison of Sepsis to Healthy Adults		
	Survivors (n = 47)	Non-survivors (n = 18)	*p*-value	Reference Interval	Below the Lower Limit	Above the Upper Limit
Naïve $CD4^+$ T cells (%)	33.77 ±16.10	21.97 ±17.62	*p*=0.012	33.020–37.257	S:22 (46.81%) N-S:13 (72.22%)	S:20 (42.55%) N-S:4 (22.22%)
Central memory $CD4^+$ T cells (%)	43.44 ±13.31	45.98 ±12.79	*p*=0.488	42.125–45.755	S:24 (51.06%) N-S:7 (38.89%)	S:22 (46.81%) N-S:8 (44.44%)
Effector $CD4^+$ T cells (%)	0.60 (0.20–2.10)	0.75 (0.27–1.55)	*p*=0.959	0.896–2.041	S:26 (55.32%) N-S:10 (55.56%)	S:12 (25.53%) N-S:3 (16.67%)
Effector memory $CD4^+$ T cells (%)	21.20 (11.60–27.50)	28.35 (13.73–41.22)	*p*=0.051	17.848–21.047	S:21 (44.68%) N-S:5 (27.78%)	S:24 (51.06%) N-S:11 (61.11%)
Naïve $CD8^+$ T cells (%)	9.50 (3.45–16.97)	5.20 (1.53–8.23)	*p*=0.061	22.550–27.557	S:39 (82.98%) N-S:16 (88.89%)	S:8 (17.02%) N-S:2 (11.11%)
Central memory $CD8^+$ T cells (%)	7.50 (3.97–15.85)	8.65 (2.38–14.48)	*p*=0.675	13.042–16.279	S:33 (70.21%) N-S:12 (66.67%)	S:10 (21.28%) N-S:3 (16.67%)
Effector $CD8^+$ T cells (%)	18.90 (10.40–32.40)	15.50 (11.68–28.10)	*p*=0.333	17.393–22.170	S:18 (38.30%) N-S:11 (61.11%)	S:20 (42.55%) N-S:6 (33.33%)
Effector memory $CD8^+$ T cells (%)	12.00 (6.60–25.70)	19.55 (7.53–46.68)	*p*=0.062	8.382–11.853	S:18 (38.30%) N-S:5 (27.78%)	S:24 (51.06%) N-S:11 (61.11%)
$CD4^+HLA\text{-}DR^+$ T cells (%)	10.60 (5.60–20.80)	16.35 (9.15–26.90)	*p*=0.131	7.846–9.894	S:16 (34.04%) N-S:4 (22.22%)	S:25 (53.19%) N-S:13 (72.22%)
$CD8^+HLA\text{-}DR^+$ T cells (%)	30.10 (22.90–45.30)	34.2 (27.75–51.87)	*p*=0.305	19.557–24.103	S:9 (19.15%) N-S:1 (5.56%)	S:34 (72.34%) N-S:15 (83.33%)
Tregs (%)	5.40 (3.50–7.30)	7.60 (5.15–9.73)	*p*=0.017	4.641–5.131	S:18 (38.30%) N-S:3 (16.67%)	S:25 (53.19%) N-S:14 (77.78%)

Note: Data are presented as means ± SD, or medians (25th - 75th percentiles).
Abbreviations: S, Survivors; N-S, Non-survivors.

count, absolute $CD8^+$ T cell count, percentage of naïve $CD4^+$ T cells, and percentage of Tregs were selected as independent variables. These were used to establish a binary logistic regression model to evaluate factors associated with death of patients with sepsis. Of the selected laboratory values, only absolute lymphocyte count and naïve $CD4^+$ T cell percentage were statistically significant in the model (Table 5).

Development of the Predictive Model for Discriminating Between Survivors and Non-Survivors of Sepsis

To generate a predictive model, ROC curve analysis of statistically significant indicators was conducted. Two indicators were identified: absolute lymphocyte count and naïve $CD4^+$ T cell percentage, with AUC >0.7 and a cut-off value of 435 cells/μL for absolute lymphocyte count and 20.25% for naïve $CD4^+$ T cell percentage, respectively. We selected these two indicators for use in a 2-Indicator Model for further multivariate analyses. The predictive model based on the combination of absolute lymphocyte count and naïve $CD4^+$ T cell percentage performed best in distinguishing the non-survival group from the survival group in patients with sepsis. This model yielded an AUC of 0.752 (95% CI, 0.602–0.901), with a sensitivity of 55.6% and a specificity of 100%; the predictive accuracy of the 2-indicator model was 80.0%. The ROC curves of single indicators and the 2-indicator model are shown in Table 6 and Figure 4.

Discussion

In this study, we reported a comprehensive analysis of T-lymphocyte subtypes in patients with sepsis, differentiating between 28-day survivors and 28-day non-survivors. Compared with survivors, absolute lymphocyte count, absolute numbers of total $CD3^+$ T cells and $CD4^+$ and $CD8^+$ T cells, as well as the percentage of naïve $CD4^+$ T cells, were significantly lower in non-survivors. The percentage of Tregs was significantly higher in non-survivors. Logistic regression analysis revealed that the absolute lymphocyte count and percentage of naïve $CD4^+$ T cells were significantly associated with sepsis outcomes.

Previous studies have shown that lymphocytes decline rapidly in the initial stage of sepsis.[22] Lymphocytopenia is a universal finding in patients with sepsis (both survivors and non-survivors), and numerous studies found it to be

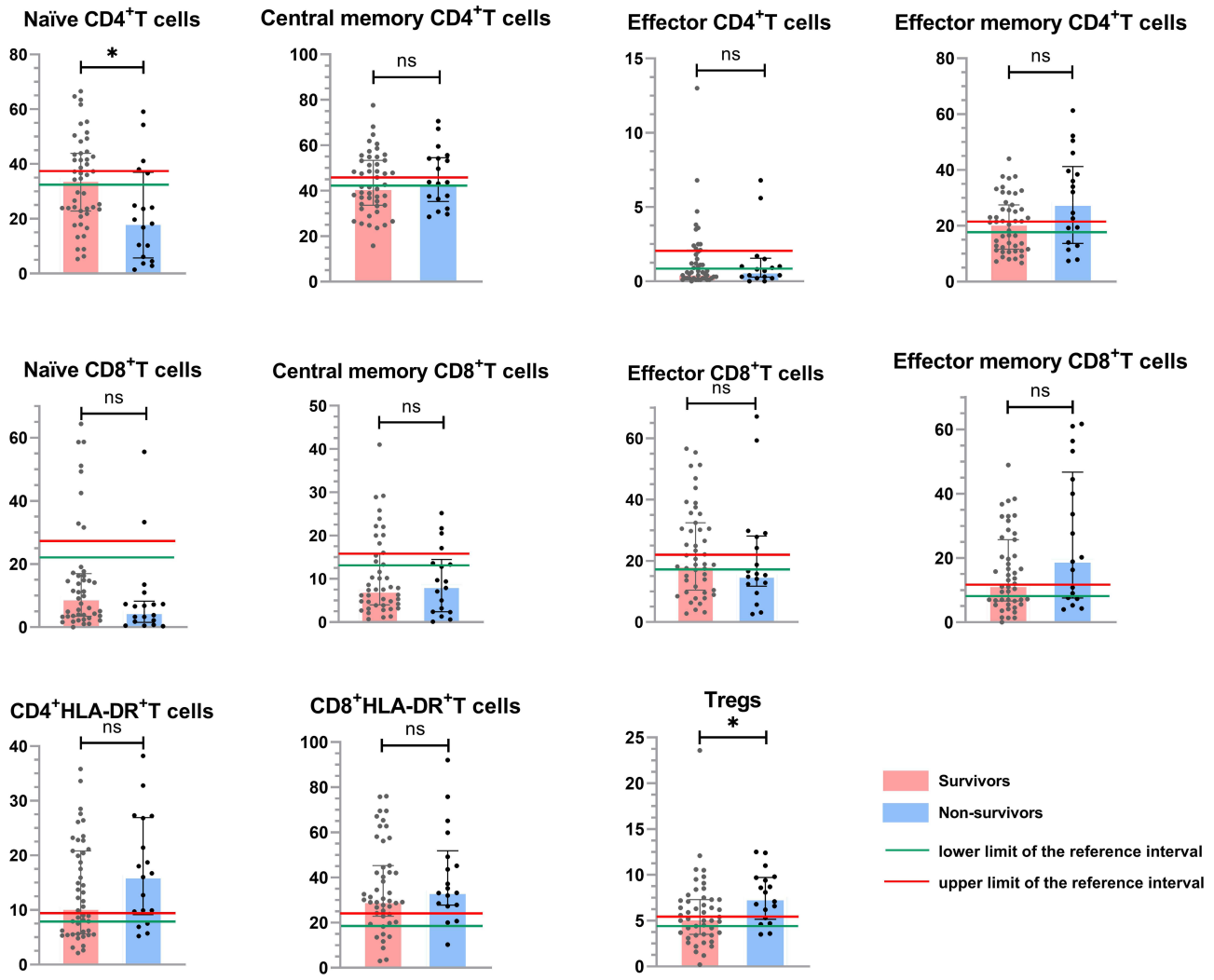

Figure 3 The percentage of T cell subpopulations in patients of sepsis with different outcomes. (survivor n=47, non-survivor n=18) *:P < 0.05, ns: not significant (Student's *t*-test or Mann–Whitney *U*-test). The boxplots depict the median, and 25th to 75th percentiles, the scatter points represent all the data values, and the horizontal lines in each graph are the lower and upper limit of the reference interval for T cell subpopulations in our lab.

associated with prognosis.[23,24] Our study confirmed this finding, with a significantly lower absolute lymphocyte count in the non-surviving group than in the surviving group. The logistic regression analysis and ROC curve showed that absolute lymphocyte count can be used as a risk factor for predicting sepsis prognosis. Total lymphocyte count is routinely obtained in clinical practice, making it a reliable indicator for monitoring sepsis progression.

Table 5 Multivariate Logistic Regression Analysis of Death in Sepsis Patients

	p-value	OR	95% CI
Lymphocyte AC	*p*=0.023	0.996	0.992–0.999
$CD3^+$ T cells AC	*p*=0.638	0.994	0.970–1.019
$CD4^+$ T cells AC	*p*=0.537	1.008	0.982–1.036
$CD8^+$ T cells AC	*p*=0.460	1.010	0.984–1.036
Naïve $CD4^+$ T cells (%)	*p*=0.030	0.949	0.906–0.995
Tregs (%)	*p*=0.399	1.073	0.887–1.350

Abbreviations: AC, absolute count, OR, odds ratio.

Table 6 Performance of Indicators and Model in Predicting Prognosis of Sepsis

	Lymphocyte AC (/μL)	$CD3^+$ T cells AC (/μL)	$CD4^+$ T cells AC (/μL)	$CD8^+$ T cells AC (/μL)	Naïve $CD4^+$ T cells (%)	Tregs (%)	2- Indicator Model
AUC	0.715 (0.562–0.868)	0.696 (0.535–0.857)	0.689 (0.529–0.850)	0.663 (0.491–0.835)	0.706 (0.556–0.857)	0.693 (0.554–0.831)	0.752 (0.602–0.901)
Cut-off value	435	262.62	179.68	80.725	20.25	6.5	0.475
Sensitivity (%)	50.0	50.0	55.6	55.6	55.6	66.7	55.6
Specificity (%)	91.5	87.2	87.2	89.4	80.9	66.0	100
PPV (%)	69.2	60.0	62.5	66.7	52.6	42.8	100
NPV (%)	82.7	82.0	83.7	84.0	82.6	83.8	78.3
Accuracy (%)	80.0	76.9	78.4	80.0	73.9	66.2	80.0

Note: The AUC is presented as Value (95% CI).
Abbreviations: AC, absolute count; AUC, area under the curve. PPV, positive predictive value; NPV, negative predictive value.

Lymphocytopenia typically includes a decrease of both T and B cells, but we found that changes of lymphocyte subsets are closely linked to the severity of sepsis. The absolute numbers of $CD3^+$, $CD4^+$ and $CD8^+$ T cells were significantly lower in the non-survival group compared with survivors, suggesting that cellular immune function decline in the early stage of sepsis may affect prognosis. Previous studies also have shown that decreased absolute $CD8^+$ T cell count is associated with increased 28-day mortality in patients with sepsis.[25,26] Decreased $CD3^+$ T cell counts were positively correlated in one study with sepsis severity and elevated mortality in patients with sepsis.[27] In our data, we found that the percentage of T-lymphocyte subsets did not differ significantly between the two groups (shown in Table 3). This finding suggests that the absolute number of lymphocyte subsets in peripheral blood may more accurately reflect the immune function state of the patient. At the early stage of sepsis, the absolute number of lymphocyte subsets in the survival group was higher; this might reflect more rapid recovery of immune function and better anti-infective activity during disease progression, improving outcomes. Some investigators have observed differences in the absolute number of

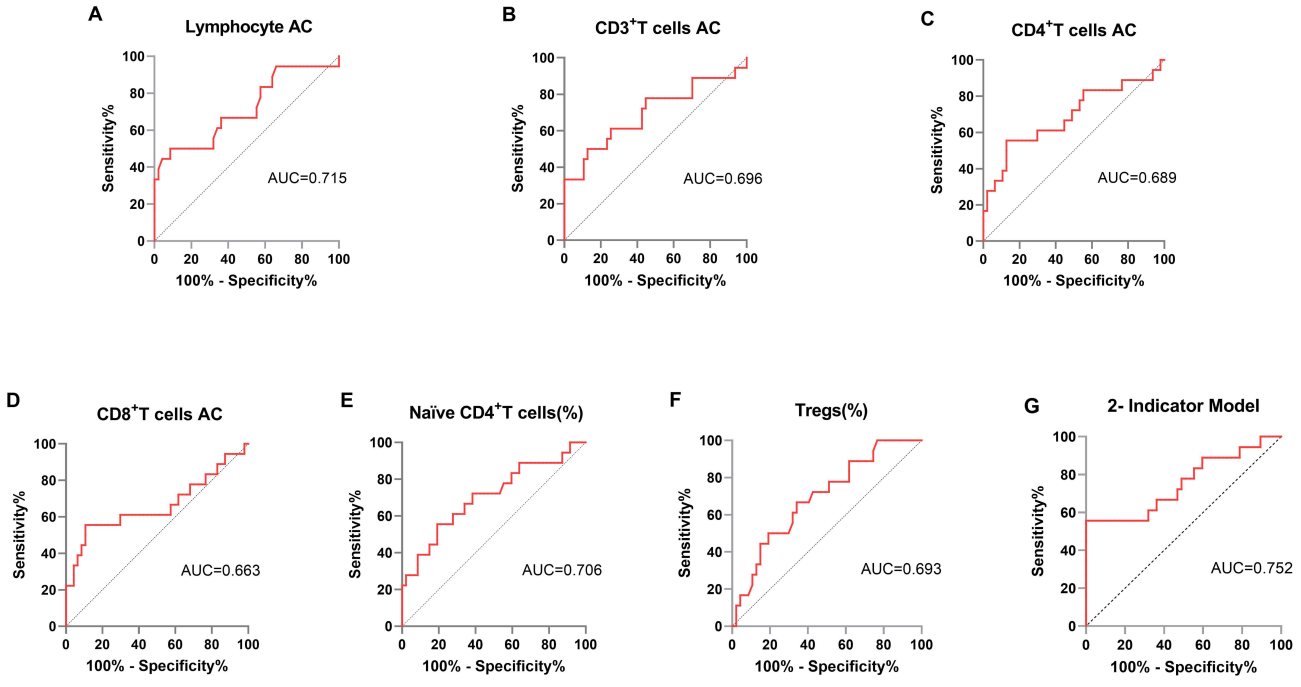

Figure 4 The receiver operating characteristic curve (ROC) of different indicators for discriminating between survivors and non-survivors of sepsis. ROC analysis showing the performance of (**A**) lymphocyte AC; (**B**) $CD3^+$T cell AC; (**C**) $CD4^+$T cell AC; (**D**) $CD8^+$T cell AC; (**E**) naïve $CD4^+$T cells (%); (**F**) Tregs (%), and (**G**) 2- Indicator Model in distinguishing survivors and non-survivors of sepsis. (survivor n=47, non-survivor n=18).
Abbreviations: ROC, receiver operating characteristic curve; AC, absolute count; AUC, area under the curve.

lymphocyte subsets between the severe group and the improved group on the first and fifth day of sepsis, with the improved group having a higher absolute number of lymphocyte subsets. On recovery, the absolute number of $CD8^+$ and $CD3^+$ T cells in the improved group significantly increased, suggesting that the immune function of the improved group was more easily restored.[28] The mechanism by which the number of lymphocytes and lymphocyte subsets in sepsis decreases is very complex, but may involve enhanced immune cell apoptosis during sepsis. Early monitoring of host immune status may alert treating clinicians to an immunosuppressed state in sepsis, permitting timely immunomodulatory therapy. The expert consensus on the monitoring and treatment of sepsis-induced immunosuppression strongly suggests that immune monitoring be initiated within 48 hours after sepsis diagnosis.[29]

We further analyzed the correlation of T-lymphocyte subpopulations with the prognosis of sepsis, observing that naïve $CD4^+$ T cell percentage was significantly lower in non-survival group, while the Treg percentage was significantly higher in non-survival group. Naïve T cells, a mature T cell in a quiescent state identified by the expression of CD45RA, can respond to antigens and performs immune surveillance by circulating between the blood and secondary lymphatic organs.[30] During early stages of sepsis, naïve T cells can affect the recovery of T cells as sepsis progresses, which may impact prognosis. Preliminary experiments assessing the recovery of $CD4^+$ T cells showed that their recovery during sepsis occurs through homeostasis proliferation of naïve cells driven by antigen, or growth of endogenous memory $CD4^+$ T cell populations.[31] Thus, it is not surprising that the group with higher percentage of naïve $CD4^+$ T cells at the initial phase of sepsis had a better prognosis. A similar difference was seen with naïve $CD8^+$ T cells, but the difference was not statistically significant, perhaps because of the small number of specimens. Tregs, a group of T-helper cells which express $CD25^+CD127^{low}$, are the central regulators of adaptive immunity, helping maintain self-tolerance by inhibiting effector T cell subpopulations.[17] According to some reports, Tregs may function not only to inhibit the proliferation and excitation of T cells, but also to hinder the function of innate immune cells.[32] In sepsis, Tregs contribute to immune paralysis by promoting T-lymphocyte apoptosis and inhibiting T-lymphocyte proliferation while promoting an anti-inflammatory state. Some scholars have reported that an increased proportion of Tregs in peripheral blood obtained from patients with severe burns has a direct influence on the occurrence and progress of sepsis. In these patients, continued high levels of Tregs should be considered an important risk factor for increased mortality risk in patients with sepsis.[33,34] This observation is consistent with our observations. This study only observed absolute lymphocyte count and the classification of immune phenotype at the initial stage of sepsis. Emerging evidence suggests that immunosuppression can occur at any time after the onset of sepsis.[35] Immune surveillance should be continuously monitored during the progression of sepsis disease, and reduced lymphocyte count, increased proportion of Tregs, and T1/T2 ratio imbalance can be used to monitor immunosuppression in patients with sepsis. Moreover, decreased lymphocyte count can be used as an initiating factor to consider immunotherapy in sepsis patients.[29] During immunotherapy for sepsis, the dynamic monitoring of lymphocyte number and subsets can help to evaluate the immune status of patients and guide the immunotherapy.[35,36]

The logistic regression analysis and the ROC curve showed that naïve $CD4^+$ T cells could be a risk factor useful for predicting the prognosis of sepsis. The AUC of Tregs was 0.693, which was inferior to that of naïve $CD4^+$ T cells in predicting prognosis. In our final model, absolute lymphocyte count combined with percentage of naïve $CD4^+$ T cells were predictive of the prognosis of sepsis, and this model had the best performance. Monitoring multiple immune parameters may be helpful for understanding the immune status and the abnormal immune response in sepsis, and for guiding initiation of immune-regulating therapy. Nevertheless, this model requires further evaluation in large samples and multi-center clinical trials.

We also observed a difference of T-lymphocyte subpopulations between patients with sepsis and healthy adults. By comparing the results of patients with sepsis to reference intervals obtained from healthy people, we found that naïve T cells and central memory T cells were lower for both $CD4^+$ T cells and $CD8^+$ T cells. However, the decrease in $CD8^+$ T cells was more obvious, suggesting an immune response to pathogens. As a significant participant in immune response, HLA-DR levels may indicate the activation state of immune cells. HLA-DR is a marker of late activation of T lymphocytes and plays a role in antigen presentation. Its expression begins to rise 24 hours after stimulation and may remain elevated for several weeks.[37,38] In our study, there was no significant difference between the non-survival

group and survival group in $CD4^+HLA\text{-}DR^+$ T cells and $CD8^+HLA\text{-}DR^+$ T cells. Both were greater than the upper limit of the reference interval, suggesting that T lymphocytes are activated in patients with sepsis.

There are several limitations to be recognized in our present study. First, regarding the model, the limited number of patients in our study could generate bias in the regression model, which requires consideration In addition, this investigation was a preliminary study with a small sample size, and no external verification of the model was carried out. In the future, a large amount of data will be studied to improve this deficiency. Second, we were not able to fully control the potential effects of underlying diseases on immune metabolism and the overlapping effects of the immune metabolism and inflammation in sepsis itself. Third, in this study, we only evaluated the changes of absolute lymphocyte count and T lymphocyte subpopulations at the early stage of sepsis. However, considerable evidence shows that the innate immune response is significantly impaired in the early stage of sepsis, and its severity and duration are also closely related to the clinical prognosis of sepsis patients. One of the hallmarks of impaired acquired immune response is lymphopenia, manifested by a decrease in both circulating T and B lymphocytes. Last, immune response of patients with sepsis may vary due to the source of infection (bacteria, virus, or other microorganisms). We did not collect data related to the source of infection, so immune analysis of this factor is lacking. We will continue to study immune function detection and evaluation of sepsis patients, collecting more immune monitoring data. We plan a comprehensive analysis of the value of neutrophils, monocytes, macrophages and adaptive immunity, including T and B lymphocytes, in the occurrence, development, and treatment of sepsis. Whether the etiology of infection can cause differences in the immune function of patients with sepsis will also be evaluated in the future.

Conclusions

Here we report the diversity of T cell subsets at the initial stage of sepsis. By comparing the differences in T cell subpopulations between patients alive at 28 days and the non-survival group, we found that absolute lymphocyte count and percentage of naïve $CD4^+$ T cells had prognostic value for survival of sepsis. The cut-off values were set at 435 cells/μL for absolute lymphocyte count and 20.25% for naïve $CD4^+$ T cells, respectively. This result suggested that T lymphocyte parameters are closely associated with sepsis prognosis. Timely, effective, and personalized immunoregulation should be given high priority in the treatment of sepsis, and methods to regulate the balance of the immune system through precise treatment at different stages of sepsis deserve close attention.

Abbreviations

APACHE II, Acute Physiology and Chronic Health Evaluation; SOFA, Sepsis-related organ failure assessment; CRP, C-reactive protein; PCT, procalcitonin; CBC, Complete blood count; NEU%, Neutrophil percentage; NEU#, Absolute neutrophil count; LYM%, Lymphocyte percentage; LYM#, Absolute lymphocyte count; NK cells, Nature killer cells; IQR, Interquartile range; AC, Absolute count; OR, Odds ratio; AUC, Area under the curve; PPV, Positive predictive value; NPV, Negative predictive value.

Data Sharing Statement

The datasets which were analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

This study has been approved by the Ethics Committee of Peking University First Hospital and has been carried out in accordance with *The Code of Ethics of the World Medical Association (Declaration of Helsinki)* for experiments involving humans and its later amendments. The ethical No. is 2023-011. Due to the retrospective nature of the study, the need for informed consent to participate was waived by the institutional review board of the Ethics Committee of Peking University First Hospital. Researchers only used the data to conduct research, and would strictly keep personal information and privacy confidential. No patients' personal information would be used in the analysis or reporting of the study results.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors declare that they have no competing interests.

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