Predictive Value of Immune Cell Subsets for Mortality Risk in Patients With Sepsis

Ying Zhang, MM, Jia Wang, MM, Le Hu, MM, Jingchao Xuan, MM, Yifan Qu, MM, Yixuan Li, MM, Xinghua Ye, MM, Long Yang, MM, Jun Yang, MM, Xiangqun Zhang, MM, Junyu Wang, MD, and Bing Wei, MM

Abstract
This study investigates the prognostic value of immune cell subsets in assessing the risk of death in patients with sepsis. This retrospective study collected 169 patients from March 2020 to February 2021 at our hospital. Baseline data were collected from patients. The absolute values (Abs) and percentages (%) of immune cell subsets for lymphocytes, T cells, CD4+ cells, CD8+, B cells, NK cells, and NKT cells were measured using flow Cytometry. Among the included patients, 43 patients were in the nonsurvivor group and 126 patients were in the survivor group. The age of patients in the nonsurvivor survivor was higher than that of survivor group patients ($P = .020$). SOFA, APACHE II, C-reactive protein, and procalcitonin were higher in the nonsurvivor group than in the survivor group (all $P$ values < .05). Multivariate regression analysis showed that lymphocytes (%) and SOFA were independent risk factors affecting patients’ prognosis. Lymphocytes (%) have the highest area under the receiver operating characteristic (ROC) curve (0.812). The model area under the ROC curve for immune cell subsets was 0.800, with a sensitivity of 72.09%, and specificity of 79.27% ($z = 7.796$, $P < .001$). Analysis of patient prognosis by immune cell subsets diagnostic showed statistically significant differences in the grouping of cut-off values for all 5 indicators (all $P < .05$). The lymphocytes (%) and SOFA score are independent risk factors affecting the prognosis of patients. A moderate predictive power for mortality in sepsis patients by immune cell subsets model.

Keywords
sepsis, immune cell subsets, mortality, diagnosis, prognosis

Introduction
The pathogenesis of sepsis involves a complex process of immune changes.1,2 The main reason for a high rate of in-hospital morbidity and mortality in patients with sepsis is related to organ failure secondary to trauma or infection. While suppressed organism immunity is one of the reasons for the deterioration of the sepsis patient’s condition.3 Further studies have shown that a single reduction in the production of inflammatory factors does not improve the organ functional impairment, reduce morbidity and mortality caused by sepsis.4 A common feature of immunosuppression in sepsis is impaired lymphocyte function and loss of immune function.5 The clearance or inactivation of antigen-specific T and B lymphocytes plays an important role in sepsis patients.6 Several studies have confirmed that sepsis occurs when there is an imbalance and dysfunction of lymphocyte subsets, with varying degrees of apoptosis of T cells, B cells, and NK cells, with a particularly pronounced decline in T cells.7,8 According to the antigens on the cell membrane surface, the primary immune cells in cellular immunity are T lymphocytes, divided into CD4+ and CD8+ cells. Under normal conditions, the circulating CD4+ T, CD8+ T cells, and CD4+/CD8+ T ratio are in the normal range. CD4+ T and CD8+ T cells in dynamic balance and mutual feedback regulation reflect to some extent the body’s cellular immune status.9 The main cells responsible for humoral immunity are B cells. Both
primary and secondary immunodeficiencies can lead to decreased humoral immune function. Humoral immunity is a rather complex and continuous process in which B cells, assisted by T cells, receive antigenic stimuli and form effector B cells and memory cells. NK cells are important immune cells of the body and have some T cell differentiation antigens. Natural killer T cells (NKT cells) are a particular subpopulation of T cells with T cell receptor TCR and NK cell receptors on their cell surface. NKT cells produce many cytokines and can exert cytotoxic effects similar to those of NK cells. In recent years, there have been many studies on the relationship between blood PCT levels and sepsis. Most studies now agree that PCT is a better predictor of sepsis, but changes in blood levels must be monitored dynamically. There are many reasons for elevated blood PCT levels in critically ill patients, including infection, postsurgery, trauma, postcardiopulmonary resuscitation, and renal insufficiency. Therefore, in ICU, predicting sepsis by blood PCT level alone often leads to misdiagnosis due to its low specificity. CRP is an antibody produced by liver tissues in the body under acute stress and is an acute temporal reactive protein, which is at a low level in healthy people. However, it is more sensitive in the early stage of disease and inflammatory response when the body is subject to tissue damage or inflammatory stimulation. When the body is subjected to tissue damage or inflammatory stimulation, CRP levels rise abruptly and change earlier than the peripheral blood leukocyte count. Furthermore, the rate and level of CRP rise are proportional to the severity of the infection and decreases as the inflammation subsides and the body’s tissue structure and function are restored. Although increased CRP levels correlate with the severity of endometrial disorders in organs of patients with sepsis, the specificity of CRP is low. Because other non-inflammatory diseases, such as burns, trauma, and major surgery, can also lead to increased CRP levels, resulting in the lack of specificity of CRP in the diagnosis of sepsis.

In recent years, changes in immune function in sepsis patients have received more extensive attention. More studies have shown that the immune system of sepsis patients is significantly suppressed. As evidenced by the suppression of T-cell number and function, and the detection of T-cell subsets has become a common examination item in ICUs. Therefore, the main purpose of this study is to investigate whether the combination of blood PCT and CRP levels and the advantages of immune cell subsets can make an early prediction of the occurrence of death in patients with sepsis.

**Materials and Methods**

**Collection of Patient Baseline Data**

In this study, a retrospective analysis was carried out to collect the medical records of 196 patients with sepsis treated at the Emergency Department ward of Beijing Chao-Yang Hospital from March 2020 to February 2021. Basic information of the study subjects was collected through the inpatient electronic information system of the authors’ hospital. Basic information included gender, age, length of stay, noninvasive ventilator, and medical history, etc. Beijing Chao-Yang Hospital’s ethics committee approved this study.

**Inclusion and Exclusion Criteria**

Inclusion criteria included (1) age ≥14 years; (2) meeting the latest diagnostic criteria of Sepsis 3.0; (3) diagnosis of sepsis within 24 h of admission to the emergency department. Exclusion criteria: (1) autoimmune diseases; (2) pregnant and lactating women; (3) long-term users of glucocorticoids or immunomodulators; (4) vital organ insufficiency; (5) chronic viral infectious diseases.

**Severity Scores of Sepsis Patients**

In this study, a total of 3 methods were used to score the severity of sepsis patients. Patients were collected within 24 h of admission to the emergency department of sequential organ failure assessment (SOFA), Acute Physiology, and SOFA score as a criterion for the diagnosis of life-threatening organ failure correlated well with mortality in patients with sepsis. In the new definition of sepsis, the application of SOFA score >2 is recommended as a diagnostic criterion for sepsis. The APACHE II scoring system comprises acute physiology score (APS), age score, and 3 components, and the final score is the sum of the 3. The theoretical maximum score is 71, and the higher the score, the more severe the disease is. The GCS score includes an eye-opening response, speech response and body movement, and the sum of the 3 scores is the coma index of the patient.

**The Detection of PCT and CRP**

The concentration of PCT was measured by immunosandwich assay using a mini VIDAS automated fluorescent immunoassay analyzer from Mérieux. The reagents used were the VIDAS BRAHMS PCT quantification kit from the company. Normal reference range of PCT was <0.5 μg/L. Serum samples from both groups to be tested were assayed using CRP antibody (465 131) provided by Beckman Coulter. The expression level of CRP was measured by the turbidimetric method with a reference range of standard values from 0 to 8 mg/L.

**Statistical Analysis**

SPSS 25.0 software was used to organize and analyze the data in this study. Also, MedCalc 19.0.4 was used to produce receiver operating characteristic (ROC) curves. The
Hosmer-Lemeshow test was used for data determination and calibration. The mean ± standard deviation was used to express the continuous data that conformed to the normal distribution. And, the median, 25%, and 75% place values were used for the data that did not conform to the normal distribution. Continuous data conforming to the normal distribution were tested using the \( t \)-test; continuous data that do not conform to a normal distribution are tested with the Wilcoxon rank-sum test. Count data were expressed as percentages, and the chi-square test was used for data processing and analysis. We used logistic regression models (ENTER methods) for the combined diagnostic model of T-cell subpopulations and B cells. Multivariate Cox regression model (excluding confounders such as sex, age, diabetes, tumor, and atherosclerotic cardiovascular disease [ACVD]) for survival analysis of each immune cell subtype grouped by cut-off value. Differences were considered statistically significant when \( P < .05 \).

## Results

### Baseline Data Analysis of Patients

A total of 169 patients with sepsis were included in this study, of which a total of 43 patients died of sepsis and 126 patients were discharged after treatment. There were 98 male patients and 71 female patients, with no statistically significant difference in gender between the 2 groups (\( P = 0.981 \)). The median age of patients discharged with sepsis was 73.0 (62.8, 83.0) and the median age of patients in the nonsurvivor was 81.0 (71.0, 85.0), with a statistically significant difference between the 2 groups (\( P = .020 \)).

### Table 1. Baseline Information in Patients With Sepsis.

| Indicator                        | Groups              | Hospital discharge group (126) | Nonsurvivor (43) | \( X^2/F \) | \( P \) |
|----------------------------------|---------------------|--------------------------------|------------------|------------|---------|
| Gender                           | Male                | 73 (57.94%)                   | 25 (58.14%)      | 0.001      | .981    |
|                                  | Female              | 53 (42.06%)                   | 18 (41.86%)      |            |         |
| Age                              |                     | 73.0 (62.8, 83.0)              | 81.0 (71.0, 85.0) | −2.331     | .020    |
| Lymphocytes (%)                  |                     | 11.2 (6.7, 16.0)               | 5.4 (3.2, 7.3)   | −6.089     | .001    |
| Lymphocytes (Abs)                |                     | 965.0 (868.0, 1340.0)          | 595.2 ± 311.4    | −5.512     | .001    |
| T cell (%)                       |                     | 65.2 ± 10.6                   | 62.4 ± 12.1      | −1.404     | .160    |
| T cell (Abs, /μL)                |                     | 650.0 (452.0, 954.8)           | 319.0 (196.0, 509.0) | −5.604     | .001    |
| CD4 + cell (%)                   |                     | 36.6 ± 10.6                   | 34.7 ± 13.0      | −1.162     | .245    |
| CD4 + cell (Abs, /μL)            |                     | 358.5 (236.8, 527.5)           | 168 (99.0, 317.0) | −4.907     | .001    |
| CD8 + cell (%)                   |                     | 24.0 (17.1, 30.8)              | 20.6 (16.4, 29.5) | −0.942     | .346    |
| CD8 + cell (Abs, /μL)            |                     | 227.0 (156.0, 331.5)           | 110.0 (63.0, 165.0) | −3.595     | .001    |
| CD4+/CD8 + cell                 |                     | 1.7 (1.0, 2.3)                | 1.5 (0.9, 2.6)   | −0.011     | .991    |
| B cell (%)                       |                     | 10.4 (6.5, 16.0)               | 11.5 (8.8, 17.7)  | −1.882     | .237    |
| NK cell (%)                      |                     | 3.0 (53.0, 183.0)              | 63.0 (32.0, 107.0) | −3.269     | .001    |
| NK (Abs, /μL)                    |                     | 17.0 (7.4, 18.6)               | 15.5 ± 8.7       | −1.641     | .101    |
| NK (Abs, /μL)                    |                     | 117.0 (61.0, 189.5)            | 90.4 ± 64.9      | −2.724     | .006    |
| NK (Abs, /μL)                    |                     | 2.1 (1.1, 4.0)                 | 2.4 (1.4, 3.8)   | −0.397     | .691    |
| NK (Abs, /μL)                    |                     | 2.0 (11.0, 40.0)               | 1.5 (7.0, 23.0)  | −3.480     | .001    |
| ACVD                             | Yes                 | 14 (11.20%)                    | 5 (11.63%)       | 0.006      | .939    |
|                                  | No                  | 111 (88.80%)                   | 38 (88.37%)      |            |         |
| T2D                              | Yes                 | 56 (44.44%)                    | 12 (27.91%)      | 3.646      | .056    |
|                                  | No                  | 70 (55.56)                     | 31 (72.09%)      |            |         |
| Tumor                            | Yes                 | 23 (18.25%)                    | 12 (27.91%)      | 1.819      | .177    |
|                                  | No                  | 103 (81.75%)                   | 31 (72.09%)      |            |         |
| Noninvasive ventilator           | Yes                 | 25 (19.84%)                    | 19 (44.19%)      | 9.867      | .002    |
|                                  | No                  | 101 (80.16%)                   | 24 (55.81%)      |            |         |
| Hospital stays                   |                     | 11 (8, 15)                     | 7 (5, 14)        | −2.074     | .038    |
| SOFA                             |                     | 4.0 (3.0, 6.0)                 | 6.0 (5.0, 9.0)   | −4.735     | .001    |
| APACHE II                        |                     | 13.0 (9.8, 16.0)               | 18.0 ± 5.8       | −4.595     | .001    |
| GCS                              |                     | 15.0 (12.0, 15.0)              | 12.0 (7.0, 15.0)  | −2.964     | .003    |
| CRP                              |                     | 24.0 (8.0, 91.7)               | 79.0 (24.0, 120.0) | −3.437     | .001    |
| PCT                              |                     | 0.05 (0.05, 0.23)              | 0.31 (0.05, 1.56) | −3.695     | <.001   |
| WBC                              |                     | 8.7 (6.4, 11.8)                | 10.5 ± 4.9       | −1.177     | .239    |
| PLT                              |                     | 210.2 ± 88.6                   | 209.0 (138.0, 250.0) | −0.433     | .665    |
| Hb                               |                     | 120.1 ± 27.2                   | 104.4 ± 29.0     | 3.208      | .002    |
| HCT                              |                     | 36.1 ± 8.05                    | 32.2 ± 8.4       | 2.704      | .008    |
| Albumin                          |                     | 31.4 ± 6.7                     | 27.2 ± 6.3       | 3.662      | <.001   |

Abbreviations: Abs, absolute value; ACVD, acute cerebrovascular disease; T2D, Type 2 diabetes mellitus; SOFA, Sequential Organ Failure Assessment; APACHE II, Acute Physiology and Chronic Health Evaluation; GCS, Glasgow Coma Scale; CRP, C-reactive protein; PCT, Procalcitonin; WBC, White Blood Cell; PLT, Platelet; Hb, Hemoglobin; HCT, Hematocrit.
groups \((P = .020)\). In terms of length of stay, the mean length of stay was 11 days for patients discharged with sepsis, while the mean length of stay was 7 days for those who died of sepsis, with a statistically significant difference between the 2 groups \((P = .038)\). Patients in the nonsurvivor group used noninvasive mechanical ventilation more than those in the survivor group, with a statistically significant difference \((P = .002)\). There was no statistical difference between the 2 groups in the occurrence of underlying diseases, such as ACVD, T2D, and tumor. Detailed results are shown in Table 1.

In the statistics of absolute values and percentages of cells in both groups, patients in the discharge group had higher lymphocytes \((\%, \text{Abs})\) T cells \((\text{Abs})\), CD4\(^+\) cells \((\text{Abs})\), CD8\(^+\) cells \((\text{Abs})\), B cells \((\text{Abs})\), NK cells \((\text{Abs})\), and NKT cells \((\text{Abs})\) than patients in the nonsurvivor survivor \((P < .001)\). The CD4\(^+\)-CD8\(^+\) values in both groups were not statistically significant \((P = .759)\) (Table 1).

In all scores of sepsis patients, the nonsurvivor group had higher SOFA and APACHE II, while had lower GCS than the survivor group, with statistically significant differences between the 2 groups \((P < .001)\). In contrast, patients in the nonsurvivor group had higher CRP and PCT than the control group, while they had lower Hb, HCT and albumin than the nondeath patient group, and the differences were statistically significant \((P < .001)\). However, there was no statistical difference in WBC and PLT between the 2 groups \((P > .05)\) (Table 1).

### The Results of Multivariate Regression Analysis

We perform Logistic regression analysis on the indicators that show meaningful results from the univariate regression analysis. The multivariate regression analysis shows the lymphocytes \((\%)\) \((P = .020)\) and SOFA \((P = .015)\) were independent influencers of patient prognosis, respectively. More detailed results are shown in Table 2.

### The Results of the ROC Curve

We performed a diagnostic analysis of the indicators of the T-cell subgroup and other indicators of death in patients with sepsis. Among the results, lymphocytes \((\%)\) had the largest AUC area \((\text{AUC} = 0.812)\). The best index of Youden index was also lymphocytes \((\%)\). The best sensitivity index of the factor is NK \((\text{Abs})\), with a sensitivity of 95.35%. The best index of specificity was CD4\(^+\) cells \((\text{Abs})\), with a specificity of 81.75% \((\text{Table 3})\). Among them, the diagnostic efficiency was higher than the patient’s age for CD8\(^+\) cells \((\text{Abs})\), lymphocytes \((\%)\) and lymphocytes \((\text{Abs})\); the rest of the indicators did not differ from the patient’s age \((P > .05)\). The diagnostic efficacy of NK \((\text{Abs})\) was higher than that of CD8\(^+\) cells \((\text{Abs})\), lymphocytes \((\%)\), and Lymphocytes \((\text{Abs})\); the diagnostic efficacy of NK \((\text{Abs})\) was not statistically different from that of other indicators \((P > .05)\). The diagnostic efficacy of lymphocytes \((\%)\) and CD8\(^+\) cells \((\text{Abs})\) was higher than that of GCS \((P < .05\), respectively\); the rest of the indicators did not differ from GCS, and the diagnostic efficacy of lymphocytes \((\%)\) was higher than that of PCT \((P < .05)\). The cut-off value of each index was calculated in this study, and the detailed results are shown in Table 3.

### Construction of the Diagnostic Model

We constructed a diagnostic model for the combination of T cell subtypes and B cells. The AUC value of the model was

### Table 2. The Results of Multivariate Regression Analysis.

| Indicator     | B    | SE   | Wald  | df | Sig. | Exp (B) | 95% CI of Exp(B) |
|---------------|------|------|-------|----|------|---------|-----------------|
| Age (years)   | 0.032| 0.023| 1.973 | 1  | 0.160| 1.033   | 0.987, 1.081    |
| Lymphocytes (%)| -0.205| 0.088| 5.414 | 1  | 0.020| 0.815   | 0.686, 0.968    |
| Lymphocytes (Abs)| 0.001| 0.003| 0.178 | 1  | 0.673| 1.001   | 0.995, 1.007    |
| T cell (Abs/μL)| 0.000| 0.006| 0.004 | 1  | 0.949| 1.000   | 0.989, 1.012    |
| CD4\(^+\) cell (Abs/μL)| -0.002| 0.005| 0.096 | 1  | 0.756| 0.998   | 0.988, 1.009    |
| CD8\(^+\) cell (Abs/μL)| -0.005| 0.006| 0.760 | 1  | 0.383| 0.995   | 0.984, 1.006    |
| B cell (Abs/μL)| -0.005| 0.005| 1.184 | 1  | 0.277| 0.995   | 0.985, 1.004    |
| NK (Abs/μL)| -0.002| 0.005| 0.158 | 1  | 0.691| 0.998   | 0.988, 1.008    |
| NKT (Abs/μL)| -0.020| 0.019| 1.102 | 1  | 0.294| 0.980   | 0.943, 1.018    |
| SOFA          | 0.294| 0.120| 5.975 | 1  | 0.015| 1.342   | 1.060, 1.699    |
| APACHE II     | 0.005| 0.079| 0.004 | 1  | 0.951| 1.005   | 0.861, 1.173    |
| GCS           | -0.046| 0.110| 0.175 | 1  | 0.676| 0.955   | 0.770, 1.185    |
| CRP           | 0.002| 0.006| 0.094 | 1  | 0.759| 1.002   | 0.990, 1.013    |
| PCT           | 0.032| 0.047| 0.456 | 1  | 0.499| 1.033   | 0.941, 1.133    |
| T2D           | -0.129| 0.568| 0.051 | 1  | 0.821| 0.879   | 0.289, 2.675    |
| Hb            | -0.047| 0.051| 0.842 | 1  | 0.359| 0.954   | 0.864, 1.054    |
| HCT           | 0.089| 0.172| 0.268 | 1  | 0.605| 1.093   | 0.780, 1.533    |
| Albumin       | -0.034| 0.048| 0.523 | 1  | 0.470| 0.966   | 0.880, 1.061    |
| Constant      | 1.444| 3.678| 0.154 | 1  | 0.695| 4.237   |                 |

Abbreviations: SE, Standard error; df, degree of freedom; CI, confidence interval; LL, lower limit; UL, upper limit; Sig., Statistically significant results.
obtained as 0.800, the Youden index was 0.515, the sensitivity was 72.09%, and the specificity was 79.27% (\(z = 7.796, P < .001\)) (Figure 1). With the constructed model, we can bring each of these 5 indicators into the equation and calculate the probability value for death in patients. Usually, when the \(P\)-value in the equation is > .5, it is presumed that the patient has a higher risk of death. The Hosmer-Lemeshow test result was 0.819, which concluded that the model was able to fit the observed data well.

### Prognosis of Patients With Cut-Off Values for Immune Cell Subtypes

We divided the 5 groups of immune cell subtypes into 2 groups according to the cut-off values of ROC curves results. The results showed statistically significant differences between the 2 groups for CD4+ cells (Abs), CD8+ cells (Abs), B cells (Abs), NK (Abs), and NKT (Abs) (all \(P\) values < .05). When we used the multivariate Cox regression model to exclude confounding factors such as sex, age, diabetes, tumor, and ACVD, there were still statistical differences in the subtypes of cells grouped using cut-off values (all \(P\) values < .05). More details results are shown in Table 4 and Figure 2.

### Discussion

The apoptosis of many lymphocytes in the physiological process of sepsis disease is closely related to immune dysfunction, which is the leading cause of inducing a clonally unresponsive state of T cells.\(^{21,22}\) It has been found that the degree of peripheral blood lymphocytopenia in sepsis patients correlates with the severity of the disease and poor prognosis.\(^{23}\) The mechanism by which the number and function of T cells are suppressed in sepsis is not well understood, and it may be that various inflammatory factors accelerate the apoptosis of T lymphocytes, inhibit their differentiation and weaken their immune function, etc.\(^{24,25}\) The change in the ratio of T cell subsets is an essential indicator of the immune function of the organism.\(^{26}\) Moreover, the occurrence and development of sepsis are closely related to the changes in the body’s immunity. Therefore, describing the relationship between patients with

#### Table 3. The Results of the ROC Curve.

| Indicator           | AUC    | SE   | 95% CI      | z     | \(P\)  | Youden | Cut-off value | Sensitivity | Specificity |
|---------------------|--------|------|-------------|-------|-------|--------|--------------|-------------|-------------|
| Age (years)         | 0.619  | 0.049| 0.541-0.693 | 2.428 | .015  | 0.231  | >77          | 62.79       | 60.32       |
| CD4+ cell (Abs, /μL)| 0.751  | 0.044| 0.679-0.814 | 5.674 | <.001 | 0.399  | ≤197         | 58.14       | 81.75       |
| CD8+ cell (Abs, /μL)| 0.786  | 0.042| 0.717-0.845 | 6.793 | <.001 | 0.513  | ≤168         | 79.07       | 72.22       |
| B cell (Abs, /μL)   | 0.667  | 0.045| 0.591-0.738 | 3.684 | <.002 | 0.302  | ≤116         | 81.40       | 48.80       |
| NK (Abs, /μL)       | 0.639  | 0.048| 0.562-0.712 | 2.921 | .004  | 0.226  | ≤181         | 95.35       | 27.20       |
| NK (Abs, /μL)       | 0.678  | 0.043| 0.602-0.748 | 4.119 | <.001 | 0.330  | ≤30          | 93.02       | 40.00       |
| Lymphocytes (%)     | 0.812  | 0.035| 0.744-0.868 | 9.032 | <.001 | 0.540  | ≤81          | 88.37       | 65.60       |
| Lymphocytes (Abs)   | 0.782  | 0.039| 0.712-0.842 | 7.294 | <.001 | 0.430  | ≤857         | 81.40       | 61.60       |
| SOFA                | 0.739  | 0.046| 0.666-0.804 | 5.173 | <.001 | 0.410  | >4           | 76.74       | 64.29       |
| APHACHE II          | 0.735  | 0.045| 0.661-0.799 | 5.198 | <.001 | 0.408  | >13          | 86.05       | 54.76       |
| GCS                 | 0.642  | 0.049| 0.565-0.714 | 2.902 | .004  | 0.273  | ≤12          | 53.49       | 73.81       |
| CRP                 | 0.675  | 0.043| 0.599-0.745 | 4.039 | <.001 | 0.296  | >11          | 90.70       | 38.89       |
| PCT                 | 0.666  | 0.045| 0.590-0.737 | 3.682 | <.001 | 0.351  | >0.14        | 60.47       | 74.60       |

Abbreviations: SE, Standard Error; AC, Associated criterion.

#### Table 4. Multivariate Cox Regression Model Results After Adjusting for Sex, Age, Diabetes, Tumor, and atherosclerotic cardiovascular disease (ACVD).

| Immune cells       | Hazard ratio (95% CI) | \(P\) |
|--------------------|-----------------------|-------|
| CD4+ cell (Abs, /μL)| 0.276 (0.145, 0.522)  | <.001 |
| CD8+ cell (Abs, /μL)| 0.225 (0.109, 0.463)  | <.001 |
| B cell (Abs, /μL)  | 0.299 (0.138, 0.649)  | .002  |
| NK (Abs, /μL)      | 0.152 (0.044, 0.521)  | .003  |
| NKT (Abs, /μL)     | 0.206 (0.037, 0.580)  | .003  |
sepsis and immune cell T-cell subsets can help to assess whether death occurs in patients with sepsis.27

During the development of sepsis, T cells have a protective role against homologous immunity. CD4+ T has a role in initiating the immune response in sepsis with various pathogenic microorganisms, promoting early pathogenic bacterial cell clearance and reducing the host bacterial burden.28 Kidney injury is one of the significant mortality factors in septic hosts, and CD4+ T reduces kidney injury.29 It has been shown that CD4+ T has a key role in limiting early bacteremia in a rat model of sepsis with cecum ligation perforation.30 CD8+ T plays a role in enhancing the pro-inflammatory response and increased mortality during the immune response to sepsis.31 One study showed that CD4+ T, CD8+ T percentage and CD4+ T/CD8+ T ratio were reduced in the sepsis nonsurvivor group compared to the sepsis survival group ($P < .05$).32 In this study, CD4+ (Abs) and CD8+ (Abs) cells showed the same trends as in the above study; however, there was no difference in CD4+/CD8+ between the 2 groups ($P = .991$). This indicates that septic patients have a low cellular immune function and that the impairment of organismal immune function is more severe in patients who died of sepsis.

Multivariate logistics regression analysis showed that the percentage of lymphocytes and SOFA score were independent risk factors for mortality in sepsis patients. In this case, for every unit increase in mortality in sepsis patients, lymphocytes (%) decreased by 0.205 units and the SOFA score increased by 0.294 units. This is similar to the findings of Faqhi et al33 in the study of patients with sepsis caused by COVID-19. Drewry et al34 shown that persistent lymphopenia on the fourth day

Figure 2. Prognosis of patients after grouping the absolute values of the 5 immune cell subtypes according to cut-off values. (A) CD4+ cell; (B) CD8+ cell; (C) B cell; (D) NK cell; (E) NKT cell.

![Graphs showing the prognosis of patients after grouping the absolute values of the 5 immune cell subtypes according to cut-off values.](image-url)
following the diagnosis of sepsis predicts early and late mortality and may serve to increase the risk of death. Early and late mortality and may serve as a biomarker for sepsis-induced immunosuppression.

Diagnostic studies showed that lymphocytes (%) had better AUC area and Youden index (0.540) among individual indices. NK (Abs) had the best sensitivity (95.35%), while CD4+ cells (Abs) had better specificity (81.75%). The diagnostic efficacy of lymphocytes (%) was better than NK cells (Abs), GCS score, and patient’s age; CD8+ cells (Abs) had better diagnostic performance than NK (Abs), GCS score, and patient’s age; lymphocytes (Abs) had better diagnostic performance than NK (Abs) and patient’s age. Subsequently, we constructed models for the absolute values of the 5 cell types of the lymphocyte subpopulation. The area under the AUC curve of the model was 0.800, with a sensitivity of 72.09% and a specificity of 79.37%. Although, the sensitivity and specificity of the model were average and the diagnostic efficacy of the model was only better than that of one indicator, NK (Abs) \( (P < 0.05) \), and was not statistically different from the other 4 indicators \( (P > 0.05) \). However, the model had the largest area under the AUC curve and the Youden index relative to the other single indicators. Bringing in the cellular values of each subtype of lymphocytes to the model, we can roughly calculate the value of the probability of death occurring in patients. The prediction of the probability of patient death allows for better-advanced prevention for critically ill patients and provides clinicians with a quantifiable regression profile for sepsis patients. We further divided each immune cell subgroup into 2 groups according to the cut-off values obtained from the diagnosis and showed that there was a statistical difference between the cut-off values of all 5 cellular indicators. This further validates the reliability of the cut-off values by using multivariate Cox regression model.

There are certain shortcomings in this study. For example, the inclusion of limited sample size may affect the conclusions to some extent; second, the patients included in this study were from the same hospital, not a multicenter study, and the conclusion drawn may not necessarily be suitable for other hospitals or institutions; third, the mechanisms regarding the appearance of immunosuppression are not very clear, and may involve, immune activation, apoptosis, and other mechanisms. Therefore, further studies are needed to verify the conclusions of this study.

Conclusion

The lymphocytes (%) and SOFA score are independent risk factors affecting the prognosis of patients. By having a moderate predictive power for mortality in sepsis patients by immune cell subsets.

Acknowledgments

This study was supported by Shijingshan District Medical Key Support Specialty Construction, and Beijing Municipal Administration of Hospitals Incubating Program (PX2018010).

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the Beijing Municipal Administration of Hospitals Incubating Program (grant no. PX2018010).

ORCID iD

Bing Wei https://orcid.org/0000-0002-9740-2086

References

1. Tong SY, Lye DC, Yahav D, et al. Effect of vancomycin or daptomycin with versus without an antistaphylococcal β-lactam on mortality, bacteremia, relapse, or treatment failure in patients with MRSA bacteremia: a randomized clinical trial. JAMA. 2020;323(6):527-537.
2. Hotchkiss RS, Colston E, Yende S, et al. Immune checkpoint inhibition in sepsis: a phase 1b randomized, placebo-controlled, single ascending dose study of antiprogrammed cell death-ligand 1 antibody (BMS-936559). Crit Care Med. 2019;47(5):632-642.
3. Hotchkiss RS, Monneret G, Payen D. Sepsis-induced immunosuppression: from cellular dysfunctions to immunotherapy. Nat Rev Immunol. 2013;13(12):862-874.
4. Fisher CJr., Agosti JM, Opal SM, et al. Treatment of septic shock with the tumor necrosis factor receptor: fc fusion protein. The soluble TNF receptor sepsis study group. N Engl J Med. 1996;334(26):1697-1702.
5. Hotchkiss RS, Monneret G, Payen D. Immunosuppression in sepsis: a novel understanding of the disorder and a new therapeutic approach. Lancet Infect Dis. 2013;13(3):259-268.
6. Blumenthal RL, Campbell DE, Hwang P, DeKruyff RH, Frankel LR, Umetsu DT. Human alveolar macrophages induce functional inactivation in antigen-specific CD4T cells. J Allergy Clin Immunol. 2001;107(2):258-264.
7. Carvelli J, Piperegloiu C, Bourenne J, et al. Imbalance of circulating innate lymphoid cell subpopulations in patients with septic shock. Front Immunol. 2019;10:2179.
8. Guo J, Tao W, Tang D, Zhang J. Th17/regulatory T cell imbalance in sepsis patients with multiple organ dysfunction syndrome: attenuated by high-volume hemofiltration. Int J Artif Organs. 2017;40(11):607-614.
9. Fang D, Zhu J. Dynamic balance between master transcription factors determines the fates and functions of CD4T cell and innate lymphoid cell subsets. J Exp Med. 2017;214(7):1861-1876.
10. Cuss AK, Avery DT, Cannons JL, et al. Expansion of functionally immature transitional B cells is associated with human-immunodeficient states characterized by impaired humoral immunity. J Immunol. 2006;176(3):1506-1516.
11. Diggins KE, Serti E, Muir V, et al. Exhausted-like CD8 + T cell phenotypes linked to C-peptide preservation in alefacept-treated T1D subjects. JCI Insight. 2021;6(3).
12. Eberl G, MacDonald HR. Selective induction of NK cell proliferation and cytotoxicity by activated NKT cells. *Eur J Immunol.* 2000;30(4):985-992.

13. Liu B, Li H, Lei Y, Zhao S, Sun M. Clinical significance of dynamic monitoring of procalcitonin in guiding the use of antibiotics in patients with sepsis in ICU. *Zhonghua Wei Zhong Bing Ji Jiu Yi Xue.* 2013;25(11):690-693.

14. Yu H, Qi Z, Hang C, Fang Y, Shao R, Li C. Evaluating the value of dynamic procalcitonin and presepsin measurements for patients with severe sepsis. *Am J Emerg Med.* 2017;35(6):835-841.

15. Guan J, Lin Z, Lue H. Dynamic change of procalcitonin, rather than concentration itself, is predictive of survival in septic shock patients when beyond 10 ng/mL. *Shock.* 2011;36(6):570-574.

16. Meyer ZC, Schreinemakers JM, de Waal RA, van der Laan L. Searching for predictors of surgical complications in critically ill surgery patients in the intensive care unit: a review. *Surg Today.* 2015;45(9):1091-1101.

17. Hurlimann J, Thorbecke G, Hochwald G. The liver as the site of C-reactive protein formation. *J Exp Med.* 1966;123(2):365-378.

18. Martin MD, Badovinac VP, Griffith TS, CD4+ T cell responses and the sepsis-induced immunoparalysis state. *Front Immunol.* 2020;11:1364.

19. Cabrera-Perez J, Condotta SA, Badovinac VP, Griffith TS. Impact of sepsis on CD4+ T cell immunity. *J Leukocyte Biol.* 2014;96(5):767-777.

20. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. *Crit Care Med.* 1985;13(10):818-829.

21. Nascimento DC, Alves-Filho JC, Sonego F, et al. Role of regulatory T cells in long-term immune dysfunction associated with severe sepsis. *Crit Care Med.* 2010;38(8):1718-1725.

22. Luan YY, Yin CF, Qin QH, et al. Effect of regulatory T cells on promoting apoptosis of T lymphocyte and its regulatory mechanism in sepsis. *J Interferon Cytokine Res.* 2015;35(12):969-980.

23. Inoue S, Suzuki-Utsunomiya K, Okada Y, et al. Reduction of immunocompetent T cells followed by prolonged lymphopenia in severe sepsis in the elderly. *Crit Care Med.* 2013;41(3):810-819.

24. Liptay S, Bachem M, Hacker G, Adler G, Debatin KM, Schmid RM. Inhibition of nuclear factor kappa B and induction of apoptosis in T-lymphocytes by salsalazine. *Br J Pharmacol.* 1999;128(7):1361-1369.

25. Guida MS, Abd El-Aal A, Kafafy Y, Salama SF, Badr BM, Badr G. Thymoquinone rescues T lymphocytes from gamma irradiation-induced apoptosis and exhaustion by modulating pro-inflammatory cytokine levels and PD-1, Bax, and Bcl-2 signaling. *Cell Physiol Biochem.* 2016;38(2):786-800.

26. Jergovic M, Smithey MJ, Nikolich-Zugich J. Intrinsic and extrinsic contributors to defective CD8+ T cell responses with aging. *Exp Gerontol.* 2018;105:140-145.

27. Hohlstein P, Gussen H, Bartneck M, Prognostic relevance of altered lymphocyte subpopulations in critical illness and sepsis. *J Clin Med.* 2019;8(3):353. Erratum: Hohlstein P. et al. *J Clin Med.* 2019;8(9).

28. Patricio P, Paiva JA, Borrego LM. Immune response in bacterial and Candida sepsis. *Eur J Microbiol Immunol (Bp).* 2019;9(4):105-113.

29. Hou YC, Wu JM, Chen KY, et al. Effects of prophylactic administration of glutamine on CD4+ T cell polarisation and kidney injury in mice with polymicrobial sepsis. *Br J Nutr.* 2019;122(6):657-665.

30. Al-Bayati HH, Alwan MJ. Immunohistopathological and immunological evaluation of listeria nanoparticle vaccine during the first and third semester in the pregnancy rat model. *Plant Archives.* 2020;20(2):2016-2025.

31. Drewry AM, Samra N, Skrupky LP, Fuller BM, Compton SM, Hotchkiss RS. Persistent lymphopenia after diagnosis of sepsis predicts mortality. *Shock.* 2014;42(5):383-391.