

# T-Lymphocyte Subtyping: A Potential Diagnostic and Prognostic Indicator of Cytomegalovirus Infection in Sepsis Patients

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## Research

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# Abstract

**Background:** This study aimed to investigate the use of T-lymphocyte subtyping as a diagnostic and prognostic marker for cytomegalovirus (CMV) infection in sepsis patients.

**Methods:** We assessed T-lymphocyte subtyping and other commonly used clinical parameters in sepsis patients upon admission to the intensive care unit (ICU), and evaluated their potential impact on diagnosis and outcomes of CMV infection.

**Results:** Among 599 sepsis patients, 82 were diagnosed with CMV infection. The 28-day mortality was significantly higher in CMV-infected than non-CMV-infected patients (20.7% vs. 9.9%). Both CD28<sup>+</sup>CD8<sup>+</sup> and CD8<sup>+</sup> T-cell counts could be combined with natural killer cell count, Acute Physiology and Chronic Health Evaluation II score, and immunoglobulin G as independent risk factors for CMV infection. Among 82 CMV-infected sepsis patients, 51 were assessed to have CMV-DNA negative conversion, while the other 31 were persistently positive for CMV DNA. The CD8<sup>+</sup> and CD28<sup>+</sup>CD8<sup>+</sup> T-cell counts were both independent risk factors for CMV-DNA negative conversion, with the latter having the better predictive ability. Lower CD28<sup>+</sup>CD8<sup>+</sup> T-cell count was associated with higher 28-day mortality in CMV-infected sepsis patients. The CMV-DNA negative conversion and 28-day mortality of CMV-infected sepsis patients could be predicted using cutoff values of 151 (74.5% sensitivity and 87.1% specificity) and 64.5 (52.9% sensitivity and 92.3% specificity) CD28<sup>+</sup>CD8<sup>+</sup> T cells/ml at ICU admission, respectively.

**Conclusions:** Sepsis patients with CMV infection had higher 28-day mortality than those without CMV infection. CD28<sup>+</sup>CD8<sup>+</sup> T-cell count was significantly higher in patients with CMV-DNA negative conversion than in those with persistently positive CMV DNA, and lower cell count was significantly associated with higher 28-day mortality. Therefore, CD28<sup>+</sup>CD8<sup>+</sup> T-cell count may be a potential marker for early diagnosis of CMV infection and outcome prediction.

**Trial registration:** Peking Union Medical College Hospital, registered at [chictr.org.cn](http://chictr.org.cn) (identifier ChiCTR-ROC-17010750)

## Introduction

According to Sepsis3.0<sup>1</sup>, host immune imbalance is the core mechanism for lethal organ dysfunction in sepsis patients, and the main reason for susceptibility to multiple pathogens<sup>2-3</sup>. Cytomegalovirus (CMV) is the most common opportunistic virus in immunocompromised hosts, such as those with organ transplantation, HIV infection, and long-term chemotherapy<sup>4-5</sup>, and CMV is also common in patients suffering from sepsis. Studies have shown that the incidence of CMV infection in sepsis patients during intensive care unit (ICU) hospitalization is as high as 15%–30%<sup>6-7</sup>. Compared with patients without CMV infection, the length of ICU stay and use of mechanical ventilation in patients with CMV infection are significantly prolonged, and overall mortality doubles<sup>8</sup>.

CMV infection has an immune basis. It is closely related to a decrease in CD4<sup>+</sup> T-cell and natural killer (NK)-cell counts in patients with HIV infection or organ transplantation<sup>9-10</sup>. Thus, is there also a correlation between host immune imbalance and CMV infection in sepsis patients? Does this immune imbalance affect the clinical diagnosis and treatment of CMV infection and even the prognosis? Currently, our knowledge of these issues is limited. Therefore, in this study, we collected data related to CMV infection in patients with sepsis. We explored the correlation between host immune function and clinical diagnosis, treatment and prognosis of sepsis, by starting with immune indicators such as T cells, immunoglobulin and complement.

## Methods

### Study population and design

Sepsis patients hospitalized in the ICU at Peking Union Medical College Hospital between February 2017 and July 2020 were assessed in this prospective study. Informed consent was obtained from all the patients involved. This study was approved by the Ethics Committee of Peking Union Medical College Hospital and registered at [chictr.org.cn](http://chictr.org.cn) (identifier ChiCTR-ROC-17010750).

Inclusion criteria were as follows: (1) age >18 years; (2) ICU stay  $\geq$ 48 h; and (3) diagnosis of sepsis. Sepsis was defined as life-threatening organ dysfunction resulting from host immune imbalance caused by infection. Infection was diagnosed by an intensivist and met the systemic inflammatory response syndrome criteria. Organ dysfunction was defined as Sequential Organ Failure Assessment (SOFA) score  $\geq$ 2 as a result of the infection<sup>1</sup>. The exclusion criteria were: (1) immunosuppression caused by organ or hematopoietic stem cell transplantation; (2) HIV infection; (3) immunosuppressive treatment prior to admission, that is, prednisone 0.5 mg/kg/day at least 2 weeks before the study, and tumor necrosis factor antagonist, methotrexate, or chemotherapy for cancer within 4 weeks of the study; (4) treatment with antiviral drugs active against CMV, such as ganciclovir or valganciclovir before admission; and (5) pregnancy or lactation.

Patients enrolled in this study underwent a thorough physical examination and all necessary tests and laboratory work were completed to determine the site of infection and the causative pathogen, and to eliminate bacterial colonization. For patients suspected of infection at multiple sites, two intensivists were required to confirm the infection foci. If no agreement could be reached, a senior intensivist was consulted for clarification.

The infection foci were confirmed according to the following conditions. Pneumonia was diagnosed clinically as new or progressive pulmonary infiltrates caused by infection with at least two manifestations as follows: fever  $>38^{\circ}\text{C}$  or hypothermia  $<36^{\circ}\text{C}$ ; leukocytosis ( $>12\,000$  cells/mm<sup>3</sup>) or leukopenia ( $<4000$  cells/mm<sup>3</sup>); and presence of newly purulent tracheal secretions and hypoxia. Bloodstream infection was defined as symptoms of infection combined with the presence of a typical or atypical pathogen in blood culture<sup>12</sup>. Abdominal infection was diagnosed as a new or progressive manifestation of peritonitis, such

as abdominal tenderness or rebound pain, accompanied by increased ascites and/or changes in its nature<sup>13</sup>. Skin and soft-tissue infection was diagnosed as soft-tissue infection accompanied by signs and symptoms of systemic toxicity<sup>14</sup>. Other infections included mediastinal infection and biliary or urinary tract infection, which have been described previously<sup>15</sup>.

The pathogens were isolated from multiple sites. Blood, body fluid, and tissue specimens obtained strictly in accordance with aseptic procedures from normally sterile sites were cultured. Blood were sampled simultaneously from two different parts at least. For collecting the sputum samples, patients were tracheal intubated. The aspirated samples met the standard for lower-tract samples of >25 white blood cells and <10 epithelial cells per low-power field of view. The tissue specimen was sampled by the surgeon for culture then the incisions were fully sterilized. Urine samples were required of midstream and obtained after placing or changing the catheter.

CMV infection was defined as detection of serum CMV DNA  $\geq 500$  copies/mL by real-time quantitative polymerase chain reaction (PCR), with or without clinical manifestations<sup>16</sup>. CMV negative conversion was defined as retesting of serum CMV DNA <500 copies/mL in patients with CMV infection during ICU/inpatient treatment<sup>17</sup>. Persistently positive CMV was defined as >500 copies/mL of serum CMV DNA. On the basis of the above definition, sepsis patients were first divided into CMV-infected and non-CMV-infected groups. CMV-infected sepsis patients were further divided into CMV-DNA negative conversion and CMV-DNA persistently positive groups.

Follow-up included length of ICU stay and in-hospital stay, ICU and in-hospital mortality, and 28-day mortality after enrollment. There was no standard treatment for critical illness, and all treatment options and drug choices were determined by the clinicians.

## **Clinical and laboratory evaluation**

### **Clinical assessments**

All patients underwent a comprehensive clinical evaluation on the day of admission to the ICU. Age, sex, underlying disease, and important infectious and biochemical indicators (Procalcitonin, creatinine, albumin and bilirubin) were all included. CMV-DNA levels were determined as well as Acute Physiology and Chronic Health Evaluation II (APACHE II)<sup>18</sup> and SOFA scores<sup>19</sup>. Life-sustaining treatments (need for mechanical ventilation, vasopressors or renal replacement therapy) for  $\geq 24$  h were recorded based on clinical evaluation and recent recommendations<sup>20</sup>. In accordance with the Third International Consensus Guidelines on the Management of Cytomegalovirus in Solid-Organ Transplantation<sup>21</sup> by the Transplantation Society International CMV Consensus Group in 2018, all patients with CMV infection received intravenous infusion of 5 mg/kg ganciclovir q12h and serum CMV-DNA levels were reviewed during treatment.

### **CMV detection**

The CMV-DNA Diagnostic Kit (Qiagen, Valencia, CA, USA) was used to detect the CMV-DNA level in plasma (per 100  $\mu$ L) by real-time quantitative PCR using Thermo-Base Taqman technology in the Roche Light Cycler 480 detection system. CMV DNA was expressed in copies/mL with a threshold of 500.

### **Immunological laboratory examination**

Blood samples were collected from each sepsis patient on the day of ICU admission for flow cytometry (Epics XL; Beckman Coulter, Brea, CA, USA) and rate nephelometry (Array 360; Beckman Coulter) to analyze the immunophenotyping of blood lymphocyte subsets and serum levels of complement factor (C)3, C4, immunoglobulin (Ig)A, IgG and IgM, as previously described<sup>22</sup>. Peripheral blood mononuclear cells were isolated and stained with combinations of different fluorescent monoclonal antibodies, followed by flow cytometric analysis (Three-Color EPICS-XL; Beckman Coulter) to detect T cells (CD3<sup>+</sup>), CD4<sup>+</sup> T-cell subsets (CD4<sup>+</sup>CD3<sup>+</sup> and CD28<sup>+</sup>CD4<sup>+</sup>), CD8<sup>+</sup> T-cell subsets (CD8<sup>+</sup>CD3<sup>+</sup>, CD28<sup>+</sup>CD8<sup>+</sup>), B cells (CD19<sup>+</sup>) and NK cells (CD3<sup>-</sup>CD16<sup>+</sup>CD56<sup>+</sup>).

### **Statistical analysis**

All analyses were performed using SPSS for Windows version 24.0 (IBM Corp., Armonk, NY, USA). Kolmogorov–Smirnov test was used to examine the cumulative distribution functions of the samples. For normally distributed continuous variables, we used Student's *t* test and analysis of variance for comparisons between two or three groups. For nonparametric variables, we chose the Mann–Whitney *U* test. The chi-square or Fisher exact test was chosen, as appropriate, for comparison of categorical variables. *P* values associated with “equal variances not assumed” were reported for variables that violated the homogeneity of variance assumption. Univariate and multivariate logistic regression analyses were performed to identify the immune parameters for prediction of CMV infection and CMV negative conversion, and the results were expressed as Wald index, *P* value and odds ratio (OR) with 95% confidence intervals (CIs). Variables that showed *P*<0.05 in univariate analysis were included in the multivariate regression analysis model, and principal component analysis was applied to adjust for the possible multicollinearity among independent variables. Variables with a variance inflation factor (VIF) >5 were considered collinear. The discriminatory ability of immune parameters for predicting 28-day mortality in sepsis patients with CMV infection was determined by receiver operating characteristic (ROC) curve analysis. The reliabilities and consistencies of diagnostic tests were assessed by calculating their sensitivity, specificity, and positive and negative predictive values. Kaplan–Meier survival analysis was used to construct survival curves, and comparisons of survival distributions were based on the log-rank test. All tests performed were two-tailed, with *P*<0.05 considered to be statistically significant.

## **Results**

### **Patients' characteristics**

During the study, a total of 637 sepsis patients were admitted to our ICU. Twenty-six were excluded according to the exclusion criteria, eight died within 48 h, two were pregnant, and two were lost to follow-

up. The remaining 599 sepsis patients were enrolled in the study (Figure 1). Tables 1 and 2 show the baseline and clinical characteristics of patients admitted to ICU. No significant differences in sex, age, underlying diseases, SOFA score, infection foci, serum biochemical parameters, and infection markers were observed between the groups. Compared with non-CMV-infected sepsis patients, the CMV-infected group had higher APACHE II and Clinical pulmonary infection scores (CPIS), a higher proportion of accompanying fungal infection, and more use of antiviral drugs. The length of ICU stay, ICU mortality, in-hospital mortality, and 28-day mortality in CMV-infected sepsis patients were also higher than those in the non-CMV-infected group. No significant difference in the length of hospital stay was found between the two groups.

### **Comparison of immune parameters in CMV- and non-CMV-infected patients**

Table 3 shows the immune parameters of all the patients at the time of enrollment. No significant differences in most immune parameters were observed between the two groups. However, NK-cell count, CD8<sup>+</sup> T-cell count, CD28<sup>+</sup> T-cell count, and IgG level in CMV-infected sepsis patients had significant differences compared with those in the non-CMV-infected group. CD8<sup>+</sup> T-cell count is the sum of CD28<sup>+</sup>CD8<sup>+</sup> and CD28<sup>-</sup>CD8<sup>+</sup> T-cell counts, multicollinearity analysis was performed for all parameters with significant differences in the univariate analysis. VIF >5 was found for the CD8<sup>+</sup> T-cell count, suggesting collinearity with other variables. Thus, we performed CD8<sup>+</sup> and CD28<sup>+</sup>CD8<sup>+</sup> T-cell counts in the multivariate logistic regression with other significantly different variables, respectively. The regression analysis showed that both CD28<sup>+</sup>CD8<sup>+</sup> and CD8<sup>+</sup> T-cell counts could be combined with NK-cell count, APACHE II score and IgG as independent risk factors for CMV infection in sepsis patients (Tables 4 and 5).

### **Comparison of immune parameters in CMV-DNA persistently positive and negative conversion patients**

To assess the effect of immune parameters on prognosis, all CMV-infected sepsis patients were further divided into CMV-DNA negative conversion and CMV-DNA persistently positive groups. Among the immune parameters, only CD8<sup>+</sup> and CD28<sup>+</sup> T-cell counts differed significantly between these two groups (Tables 7 and 8). To avoid statistical error caused by multicollinearity, CD8<sup>+</sup> and CD28<sup>+</sup>CD8<sup>+</sup> T-cell counts were similarly performed in logistic regression, respectively. Regression analysis suggested that CD28<sup>+</sup>CD8<sup>+</sup> and CD8<sup>+</sup> T cell counts were independently positively related with CMV-DNA negative conversion (OR: 1.025 vs 1.005; P<0.001 vs P=0.002). ROC analysis showed that compared with CD8<sup>+</sup> T-cell count, CD28<sup>+</sup>CD8<sup>+</sup> T-cell count had higher discriminatory power with an area under the curve of 0.898. A CD28<sup>+</sup>CD8<sup>+</sup> T-cell count cutoff of 151 cells/mm<sup>3</sup> at ICU admission may predict CMV-DNA negative conversion with a sensitivity of 74.5% and specificity 87.1% (Figure 2 and Table 9).

### **Comparison of parameters in surviving and non-surviving CMV-infected sepsis patients**

We divided all CMV-infected sepsis patients into survivor and non-survivor groups based on their 28-day mortality after enrollment. CD8<sup>+</sup> and CD28<sup>+</sup> T-cell counts showed significant differences, while other

immune parameters showed no significant differences between the two groups (Table 10). According to logistic regression analysis, CD28<sup>+</sup>CD8<sup>+</sup> and CD8<sup>+</sup> T-cell counts were independently negatively correlated with 28-day mortality after enrollment (Tables 11 and 12). Similarly, ROC curve analysis was conducted to compare the predictive efficacy of the two parameters. The CD28<sup>+</sup>CD8<sup>+</sup> T-cell count may have been more accurate in predicting 28-day mortality after enrollment for CMV-infected sepsis patients. A lower CD28<sup>+</sup>CD8<sup>+</sup> T-cell count may have been associated with a higher 28-day mortality. The sensitivity and specificity of a cutoff value of 64.5 cells/mm<sup>3</sup> in predicting the 28-day mortality of CMV-infected sepsis patients were 52.9% and 92.3%, respectively (Figure 3 and Table 13). Kaplan–Meier analysis showed that CMV-infected sepsis patients with CD28<sup>+</sup>CD8<sup>+</sup> T-cell count <64.5 cells/mm<sup>3</sup> (log-rank P<0.001) were associated with a lower survival rate in the ICU (Figure 4).

## Discussion

To our knowledge, this is the first prospective study to explore the relationship between host immune function and prognosis (including CMV negative conversion and 28-day mortality) in sepsis patients with CMV infection. Compared with patients without CMV infection, ICU, in-hospital and 28-day mortalities were significantly increased in CMV-infected sepsis patients. Both CD28<sup>+</sup>CD8<sup>+</sup> and CD8<sup>+</sup> T-cell counts could be combined with NK-cell count, APACHE II score and IgG as independent risk factors for CMV infection in sepsis patients. CD28<sup>+</sup>CD8<sup>+</sup> T-cell count was the best predictor of CMV-DNA negative conversion potential in CMV-infected sepsis patients. A CD28<sup>+</sup>CD8<sup>+</sup> T-cell count cutoff of 151 cells/mm<sup>3</sup> at ICU admission may predict the CMV-DNA negative conversion with a sensitivity of 74.5% and specificity 87.1%. A lower CD28<sup>+</sup>CD8<sup>+</sup> T-cell count may be associated with a higher 28-day mortality. The sensitivity and specificity of a CD28<sup>+</sup>CD8<sup>+</sup> T-cell count cutoff value of 64.5 cells/mm<sup>3</sup> in predicting the 28-day mortality of CMV-infected sepsis patients were 52.9% and 92.3%, respectively. Our findings reflect the important role of host immune function in sepsis patients with CMV infection and provide evidence that evaluation of systemic levels of some key immune parameters has clinical potential in predicting early diagnosis and prognosis of CMV infection. A previous study<sup>23</sup> has shown that after CMV infection, the host innate immune system, specific NK cells, multiple antibodies and effector/memory T-cell responses are activated rapidly by viruses. These responses block the continuous replication of CMV DNA, and then CD8<sup>+</sup> T cells are recruited to the infected site to eliminate the virus by releasing cytokines, which is the first stage of host immunity against CMV<sup>24</sup>. We found that NK-cell count, IgG and CD28<sup>+</sup>CD8<sup>+</sup> T-cell count in CMV-infected sepsis patients were significantly higher than in patients without CMV infection, suggesting that host immune function plays an important role in CMV infection. However, our results differ from the study of Kaplan *et al.*<sup>5</sup>, who found that CD4<sup>+</sup> T-cell count in HIV-infected patients could predict CMV infection. We did not find a significant difference in CD4<sup>+</sup> T-cell count between CMV-infected and non-CMV-infected sepsis patients. The reason for this difference may be related to the different patients in the two studies. CD4<sup>+</sup> T cells are the only specific target of HIV, and CD4<sup>+</sup> T-cell count of HIV-infected patients is closely related to susceptibility to opportunistic pathogens. Therefore, CD4<sup>+</sup> T-cell count could specifically represent the immune status of HIV-infected patients<sup>25</sup>. Our study involved

sepsis patients, in whom immune status was affected by various factors such as underlying diseases and treatment. Moreover, a study analyzing cellular immunity after CMV infection by Radha *et al.*<sup>26</sup> showed that, although CMV infection was mainly controlled by CD8<sup>+</sup> and CD4<sup>+</sup> T cells, CD8<sup>+</sup> T-cell response seemed to play a more important role in this process. By comparing T-cell proliferation after CMV infection, they found that under the same antiviral treatment, patients with rapid proliferation of CD8<sup>+</sup> T cells had faster CMV-DNA negative conversion.

The anti-CMV activity of CD8<sup>+</sup> T cells is closely related to their high sensitivity to CMV peptide fragments. It has been reported that approximately 30% of CD8<sup>+</sup> T cells in healthy adults are CMV reactive<sup>27</sup>. After the invasion of CMV, CD8<sup>+</sup> naïve T cells are rapidly amplified and differentiate into different effector cells under the joint stimulation of CMV-specific polypeptides such as CMV tegument protein 65 (PP65) and immediate-early protein 1 (IE1)<sup>28</sup>, and co-stimulators such as CD28 and CD137<sup>29</sup>, and cytokines such as interleukin (IL)-2 and IL-15<sup>30-31</sup>. These effector cells then circulate into the tissues and perform antiviral functions through perforin- and interferon-g-mediated mechanisms<sup>32</sup>. As an important co-stimulator of T cells, CD28 is involved in the activation and differentiation of CD8<sup>+</sup> T cells and is a typical surface marker that combines with others to classify functional subsets<sup>33-34</sup>. The co-stimulative effect of CD28 accelerates the response speed of T cells and is essential for host resistance to life-threatening acute viral diseases<sup>35-36</sup>. A study by Dolfi and colleague in 2016 has demonstrated that CD28<sup>+</sup>CD8<sup>+</sup> T cells have good predictive value for the incidence of CMV infection in kidney transplantation patients<sup>37</sup>. Patients with lower CD28<sup>+</sup>CD8<sup>+</sup> T-cell count have a higher incidence of CMV infection after kidney transplantation surgery, while for CMV-infected kidney transplantation patients, the increase of CD28<sup>+</sup>CD8<sup>+</sup> T-cell count is closely related to progression of CMV infection. Additionally, it has been demonstrated that expression of CD28 on the surface of T cells is significantly decreased in patients with severe infection, and the reduction of CD28 expression may be an independent risk factor for high mortality<sup>38</sup>. Our findings are consistent with the above conclusions. First, by comparing the immune parameters of all sepsis patients, we found that CD28<sup>+</sup>CD8<sup>+</sup> T cells may be an independent risk factor for CMV infection in sepsis patients. We also found that, among all CMV-infected sepsis patients, those with lower CD28<sup>+</sup>CD8<sup>+</sup> T-cell count also had less CMV-DNA negative conversion and a higher 28-day mortality after enrollment. Lastly, logistic regression and ROC curve analysis showed that CD28<sup>+</sup>CD8<sup>+</sup> T-cell count was a better predictor of prognosis than CD8<sup>+</sup> T-cell count for CMV-infected sepsis patients. These results indicate that CD28<sup>+</sup>CD8<sup>+</sup> T cells may play an irreplaceable and important role in host resistance to CMV, providing new evidence for better evaluation of prognosis in CMV-infected sepsis patients.

CD28<sup>-</sup> CD8<sup>+</sup> T cells also play an important role in host resistance to CMV infection<sup>39</sup>. Studies have demonstrated that CD28<sup>-</sup>CD8<sup>+</sup> T cells continue to expand when hosts are chronically exposed to CMV, providing lasting defense as effector memory cells<sup>40</sup> and suppressing the excessive T regulatory cell response to CMV<sup>41</sup>, to maintain immune balance. This is the main reason why CMV could be latent for the lifetime of humanity<sup>42</sup>. Therefore, CD28<sup>-</sup>CD8<sup>+</sup> T cells are currently considered to play a major role in

the second and third stages of host resistance to CMV infection<sup>24</sup>. Although CMV-infected patients were all at the CMV-DNA replication stage, we did not further distinguish the host immune phase in resistance to CMV infection. This may be one of the reasons for there being no difference in CD28<sup>-</sup>CD8<sup>+</sup> T-cell count between the groups. Another explanation for the lack of difference in CD28<sup>-</sup>CD8<sup>+</sup> T-cell count between the groups in our study is age. Numerous studies<sup>43-45</sup> have indicated that CD28<sup>-</sup>CD8<sup>+</sup> T cells are associated with immune senescence, and the proportion of CD8<sup>+</sup> T cells lacking CD28 surface expression increases with age, which may explain the increased susceptibility in elderly individuals. However, in our study, there was no significant difference in age between the groups.

Our study had several limitations. First, CMV-specific CD8<sup>+</sup> and CD4<sup>+</sup> T cells were not selected for subpopulation analysis because the purpose of this study was to explore the role of host immune function in CMV-infected sepsis patients. Thus, we selected the most common and easily available immune indicators for analysis. Second, because both initial CMV infection and reactivation are included in CMV infection, we did not distinguish the onset type of CMV infection. Third, we did not select multiple time nodes to longitudinally analyze the possible correlation between host immune function at different stages of sepsis and CMV infection. In the future, we plan to group patients according to CMV initial infection/reactivation, analyze host immune function of CMV-specific T cells, and collect data from multiple time nodes to explore the relationship between CMV infection and host immune function of sepsis patients.

## Conclusion

We found that CD28<sup>+</sup>CD8<sup>+</sup> T-cell count plays a potential role in prediction of CMV infection, CMV-DNA negative conversion and 28-day mortality in sepsis patients. Higher CD28<sup>+</sup>CD8<sup>+</sup> T-cell count is associated with incidence of CMV infection and lower 28-day mortality in sepsis patients. Our findings highlight the important role of host immune function in CMV-infected sepsis patients and provide strong evidence that T-lymphocyte subtyping could facilitate early diagnosis and prognosis of CMV infection and help identify sepsis patients with a high mortality risk.

## Abbreviations

1. **CMV** cytomegalovirus
2. **HIV** human immunodeficiency virus
3. **ICU** intensive care unit
4. **NK** natural killer
5. **SOFA** Sequential Organ Failure Assessment
6. **PCR** polymerase chain reaction
7. **APACHE II** Acute Physiology and Chronic Health Evaluation II
8. **C3** complement factor 3

9. **C4** complement factor 4
10. **IgA** immunoglobulin A
11. **IgG** immunoglobulin G
12. **IgM** immunoglobulin M
13. **OR** odds ratio
14. **CIs** confidence intervals
15. **VIF** variance inflation factor
16. **ROC** receiver operating characteristic
17. **CPIS** Clinical pulmonary infection scores
18. **PP65** CMV tegument protein 65
19. **IE1** immediate-early protein 1
20. **IL-2** interleukin-2
21. **IL-15** interleukin-15
22. **COPD** chronic obstructive pulmonary disease
23. **NG** neutrophilegranulocyte
24. **WBC** white blood cell count
25. **AUC** area under the curve

## Declarations

### Ethics approval and consent to participate

This study was approved by the local institutional review board of PUMCH (No. of Ethics approval: JS-1170). Informed consent was obtained from all the patients involved.

### Consent for publication

Not applicable.

### Competing interests

All authors declare that they have no conflicts of interest.

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### Authors' contributions

Guangxu Bai designed the study and prepared the drafting of this article. Na Cui and Hao Wang conceived the study and made final approval of the manuscript. Guangxu Bai and Jianwei Chen made analysis of all data and helped revise this manuscript. Wen Han and Wei Cheng contributed to the acquisition of laboratory data and Guangxu Bai was in charge of acquisition of clinical data.

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## Availability of data and material

The data set supporting the results of this article is included within the article.

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## Tables

**Table 1. Baseline characteristics of the study population**

Variables	Sepsis (n=599)	CMV-DNA+ (n=82)	CMV-DNA- (n=517)	P value
Age (years)	63(20)	66 (17)	63 (20)	0.204
Sex (male: female)	376:223	55:27	321:196	0.386
<b>Underlying disease (n (%))</b>				
COPD	20 (3.3)	4 (4.9)	16 (3.1)	0.404
Heart failure	177 (29.5)	25 (30.5)	152 (29.4)	0.841
Diabetic mellitus	158 (26.4)	28 (34.1)	130 (25.1)	0.086
Liver Cirrhosis	16 (2.7)	2 (2.4)	14 (2.7)	0.888
Tumor	117 (19.5)	10 (12.2)	107 (20.7)	0.071
Chronic renal failure	60 (10.0)	7 (8.5)	53 (10.3)	0.631
APACHE II score	17 (9)	18.5 (8)	16 (10)	0.000
SOFA score	11 (6)	11 (5)	11 (6)	0.534

Data are number of patients (%) or median and interquartile range. *APACHE II* Acute Physiology And Chronic Health Evaluation II; *CMV* cytomegalovirus; *COPD* chronic obstructive pulmonary disease; *SOFA* Sequential Organ Failure Assessment.

**Table 2. Clinical characteristics of the sepsis patients**

Variables	Sepsis (n=599)	CMV-DNA+ (n=82)	CMV-DNA- (n=517)	P value
<b>Life-sustaining treatments (n (%))</b>				
Mechanical ventilation	547 (91.3)	80 (97.6)	488 (94.4)	0.229
Need for vasopressor	541 (88.5)	73 (89.3)	457 (88.4)	0.868
Need for RRT	140 (23.4)	17 (20.7)	123 (23.8)	0.543
<b>Sites of infection (n (%))</b>				
Lung	274 (45.7)	32 (39.0)	242 (46.8)	0.189
Abdominal	80 (13.4)	7 (8.5)	73 (14.1)	0.167
Bloodstream	76 (12.7)	11 (13.4)	65 (12.5)	0.831
Skin& Soft tissue	30 (5.0)	3 (3.7)	27 (5.2)	0.546
Others	9(1.5)	1 (1.2)	8 (1.5)	0.821
<b>Multiple site infection (n (%))</b>				
Lung+ Blood	53 (9.5)	11 (13.4)	42 (8.1)	0.117
Lung+ Abdominal	39 (6.5)	8 (9.8)	31 (6.0)	0.200
Others	38 (6.3)	9 (11.0)	29 (5.6)	0.064
<b>Pathogens (n (%))</b>				
Bacteria	495 (83)	68 (83.1)	427 (82.9)	0.941
Fungal	73 (12.2)	16 (19.5)	57 (11.0)	0.029
Else	37 (6.2)	6 (7.3)	31 (6.0)	0.644
<b>Drug therapy (n (%))</b>				
Antifungal drugs	112 (18.7)	21 (25.6)	91 (17.6)	0.084
Antiviral drugs	107 (17.9)	82 (100)	25 (4.8)	0.000
<b>Biochemical parameters at ICU admission</b>				
Albumin (g/L)	32 (6)	32(7)	32(6)	0.557
Serum Creatinine (µmol/L)	86 (90)	95.5(80)	85(93)	0.058
Total Bilirubin (µmol/L)	17.6(13.2)	19.9(39)	17.4(11.2)	0.156
<b>Infection marker at ICU admission</b>				
Procalcitonin (ng/mL)	2.3(7.0)	1.79(5.11)	2.45(7.32)	0.12
CPIS Score	6(4)	6.5(2)	6(4)	0.036
<b>Outcome</b>				
ICU durations (days)	10(13)	16.5(22)	9(11)	0.000
Hospital durations (days)	20(23)	23(23)	19(23)	0.114
ICU mortality (n(%))	71(11.9)	17(20.7)	54(10.4)	0.007
Hospital mortality (n(%))	84(14)	19 (23.2)	65 (12.6)	0.010
28 days mortality (n(%))	68 (11.4)	17 (20.7)	51 (9.9)	0.004

Data are number of patients (%) or median and interquartile range. *CMV* cytomegalovirus; *CPIS* Clinical pulmonary infection scores; *ICU* intensive care unit.

**Table 3. Immune parameters between patients with or without CMV infection**

Variables	Sepsis (n=599)	CMV-DNA+ (n=82)	CMV-DNA- (n=517)	P value <sup>1</sup>
WBC (10 <sup>9</sup> /L)	11.79 (8.9)	10.45 (8.31)	12.07 (9)	0.112
NG (10 <sup>9</sup> /L)	9.95 (8.27)	8.37 (7.50)	10.04 (8.63)	0.154
NK (cells/mm <sup>3</sup> )	74 (85)	59.5 (54)	76 (90)	0.002
B cells	106 (119)	96 (87)	106 (123)	0.071
Lymphocyte (cells/mm <sup>3</sup> )	927 (616)	901 (532)	927 (629)	0.891
CD4+T	363 (339)	399 (352)	358 (338)	0.471
CD28+CD4+T	337 (342)	358.5 (384)	331 (333)	1.000
CD28- CD4+T	10 (24)	10 (55)	10 (24)	0.282
CD8+T	278 (280)	361.5 (241)	266 (311)	0.011
CD28+CD8+T	99 (133)	148.5 (226)	96.5 (93)	0.000
CD28- CD8+T	172 (138)	158.5 (165)	153 (212)	0.847
<b>Complement factor (g/L)</b>				
C3	0.78 (0.40)	0.79 (0.40)	0.78 (0.41)	0.632
C4	0.16 (0.10)	0.17 (0.1)	0.16 (0.10)	0.766
<b>Immunoglobulin (g/L)</b>				
IgA	2.16 (1.29)	2.21 (1.76)	2.11 (1.30)	0.837
IgG	9.66 (5.76)	11.04 (8.64)	9.24 (5.31)	0.026
IgM	0.72 (0.52)	0.74 (0.56)	0.68 (0.53)	0.923

Data are median and interquartile range. *CMV* cytomegalovirus; *NG* neutrophile granulocyte; *NK* natural killer cell; *WBC* white blood cell count.

**Table 4. Multivariate logistic regression analysis of factors distinguishing CMV infection**

Parameters	OR	95%CI	P value <sup>1</sup>
Fungal infection	0.538	0.278-1.042	0.066
CPIS	1.093	0.992-1.204	0.071
APACHE II	1.046	1.009-1.085	0.014
NK	0.992	0.988-0.997	0.001
IgG	1.074	1.026-1.124	0.002
CD28+CD8+T cell counts	1.004	1.002-1.006	0.000

*APACHE II* Acute Physiology and Chronic Health Evaluation II; *CI* confidence interval; *NK* natural killer cell; *OR* odds ratio.

**Table 5. Multivariate logistic regression analysis of factors distinguishing CMV infection**

Parameters	OR	95%CI	P value <sup>1</sup>
Fungal infection	0.563	0.294-1.076	0.082
CPIS	1.090	0.991-1.199	0.074
APACHE II	1.048	1.011-1.086	0.010
NK	0.992	0.988-0.997	0.001
IgG	1.073	1.025-1.123	0.002
CD8+T cell counts	1.001	1.000-1.002	0.008

*APACHE II* Acute Physiology And Chronic Health Evaluation II; *CI* confidence interval; *NK* natural killer cell; *OR* odds ratio.

**Table 6. Immune parameters between CMV-DNA persistently positive and negative conversion patients**

Variables	CMV-DNA+ (n=82)	Negative Conversion (n=51)	Persistently positive (n=31)	P value
WBC (10 <sup>9</sup> /L)	10.45 (8.2)	9.90 (8.08)	10.87 (7.62)	0.257
NG (10 <sup>9</sup> /L)	8.37 (7.44)	8.12 (7.91)	8.67 (7.56)	0.202
NK (cells/mm <sup>3</sup> )	60.5 (57)	59 (64)	61 (64)	0.852
B cells (cells/mm <sup>3</sup> )	101 (91.25)	93 (73)	113 (101)	0.579
Lymphocyte (cells/mm <sup>3</sup> )	891 (538.25)	990 (538)	819 (508)	0.115
CD4+T	377 (352.75)	380 (333)	418 (382)	0.793
CD28+CD4+T	351.5 (387.5)	377 (399)	301 (400)	0.488
CD28- CD4+T	10 (54.25)	7 (29)	12 (109)	0.322
CD8+T	328 (224.75)	371 (230)	240 (194)	0.000
CD28+CD8+T	153.5 (171)	216 (141)	77 (87)	0.000
CD28- CD8+T	173.5 (111.75)	165 (110)	178 (112)	0.629
<b>Complement factor (g/L)</b>				
C3	0.79 (0.40)	0.72 (0.37)	0.86 (0.34)	0.118
C4	0.17 (0.1)	0.15 (0.10)	0.19 (0.08)	0.297
<b>Immunoglobulin (g/L)</b>				
IgA	2.29 (1.47)	2.15 (1.43)	2.39 (2.11)	0.688
IgG	10.6 (8.06)	11.42 (7.55)	10.6 (13.64)	0.789
IgM	0.76 (0.56)	0.75 (0.60)	0.73 (0.55)	0.322

Data are median and interquartile range. *CMV* cytomegalovirus; *NG* neutrophile granulocyte; *NK* natural killer cell; *WBC* white blood cell count.

**Table 7. Multivariate logistic regression analysis of CD8<sup>+</sup> T-cell count distinguishing CMV-DNA negative conversion**

Parameters	OR	95%CI	P value <sup>1</sup>
CD8+T cell counts	1.005	1.002-1.009	0.002

*CI* confidence interval; *OR* odds ratio.

**Table 8. Multivariate logistic regression analysis of CD28<sup>+</sup>CD8<sup>+</sup> T-cell count distinguishing CMV-DNA negative conversion**

Parameters	OR	95%CI	P value <sup>1</sup>
CD28+CD8+T cell counts	1.025	1.014-1.036	0.000

*CI* confidence interval; *OR* odds ratio.

**Table 9. Receiver operating characteristic curve analysis of parameters predicting CMV-DNA persistently positive or negative conversion**

Parameters	Cutoff Value	AUC	95%CI	P value
CD8+T (cells/mm <sup>3</sup> )	358	73.5	0.627-0.844	0.000
CD28+CD8+T (cells/mm <sup>3</sup> )	151	89.8	0.834-0.961	0.000

*AUC* area under the curve.

**Table 10. Immune parameters in all CMV infection patients according to 28-day mortality**

Variables	CMV (n=82)	Survivors (n=59)	Non-survivors (n=23)	P value
WBC (10 <sup>9</sup> /L)	10.45 (8.2)	10.31 (8.03)	10.59 (11.47)	0.194
NG (10 <sup>9</sup> /L)	8.37 (7.44)	8.32 (6.99)	8.41 (10.49)	0.255
NK (cells/mm <sup>3</sup> )	60.5 (57)	59 (60)	61 (49)	0.895
B cells	101 (91.25)	97 (85)	95 (149)	0.864
Lymphocyte (cells/mm <sup>3</sup> )	891 (538.25)	945 (532)	774 (377)	0.085
CD4+T	377 (352.75)	380 (401)	418 (288)	0.503
CD28+CD4+T	351.5 (387.5)	365 (385)	325 (400)	0.158
CD28- CD4+T	10 (54.25)	8 (38)	57 (170)	0.169
CD8+T	328 (224.75)	358 (221)	228 (168)	0.013
CD28+CD8+T	153.5 (171)	189 (170)	64 (102)	0.000
CD28- CD8+T	173.5 (111.75)	177 (103)	142 (70)	0.417
Complement factor (g/L)				
C3	0.79 (0.40)	0.80 (0.45)	0.71 (0.20)	0.615
C4	0.17 (0.1)	0.18 (0.11)	0.16 (0.07)	0.415
Immunoglobulin (g/L)				
IgA	2.29 (1.47)	2.25 (1.58)	1.76 (2.8)	0.351
IgG	10.6 (8.06)	11.42 (8.13)	10.6 (13.38)	0.877
IgM	0.76 (0.56)	0.74 (0.56)	0.73 (0.67)	0.918

CMV cytomegalovirus; NG neutrophile granulocyte; NK natural killer cell; WBC white blood cell count.

**Table 11. Multivariate logistic regression analysis of CD28<sup>+</sup>CD8<sup>+</sup> T-cell count distinguishing 28-day mortality**

Parameters	OR	95%CI	P value
CD28+CD8+T cell counts	0.990	0.983-0.997	0.007

CI confidence interval; OR odds ratio.

**Table 12. Multivariate logistic regression analysis of CD8<sup>+</sup> T-cell count distinguishing 28-day mortality**

Parameters	OR	95%CI	P value
CD8+T cell counts	0.996	0.992-1.000	0.036

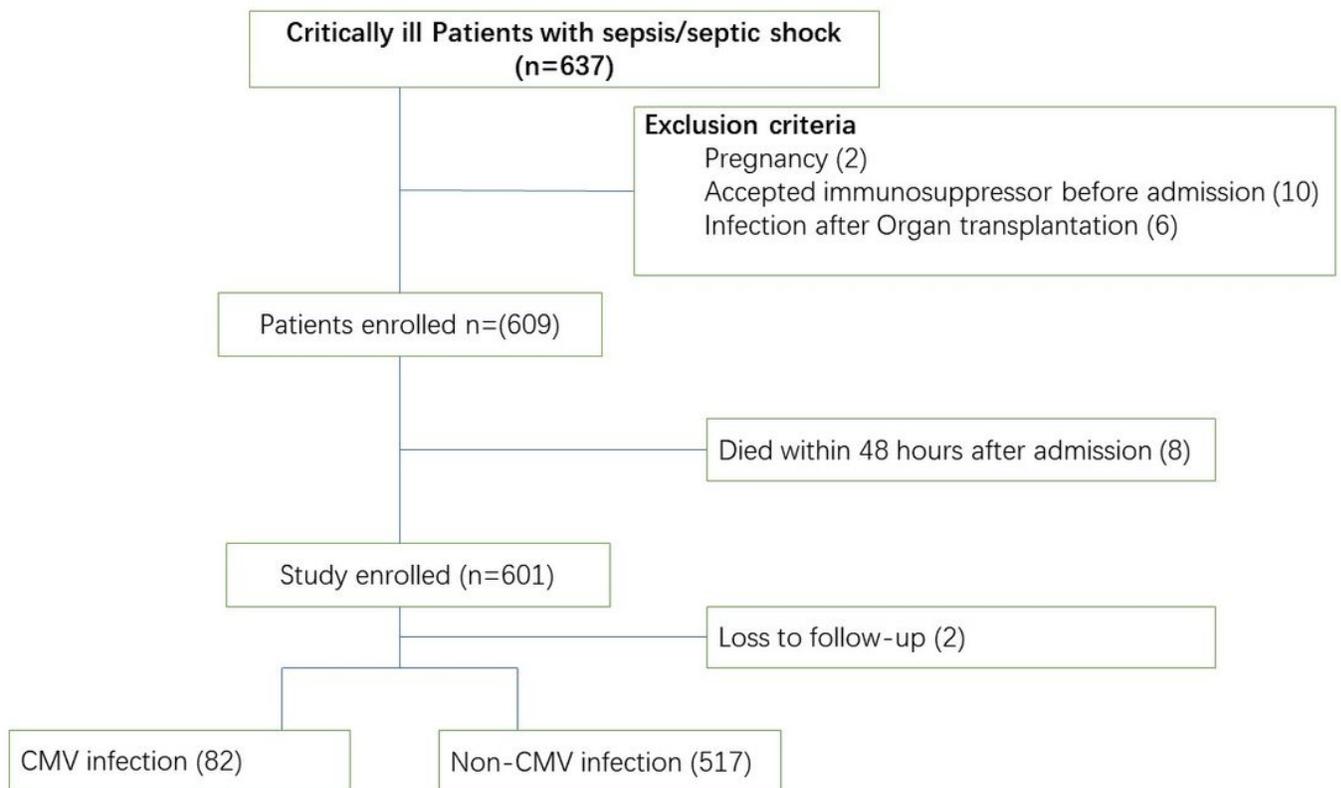
CI confidence interval; OR odds ratio.

**Table 13. Receiver operating characteristic curve analysis of parameters predicting 28-day mortality**

Parameters	Cutoff Value	AUC	95%CI	P value
CD8+T cells/mm <sup>3</sup>	266	69.7	0.555-0.839	0.013
CD28+CD8+T cells/mm <sup>3</sup>	64.5	76.2	0.627-0.897	0.001

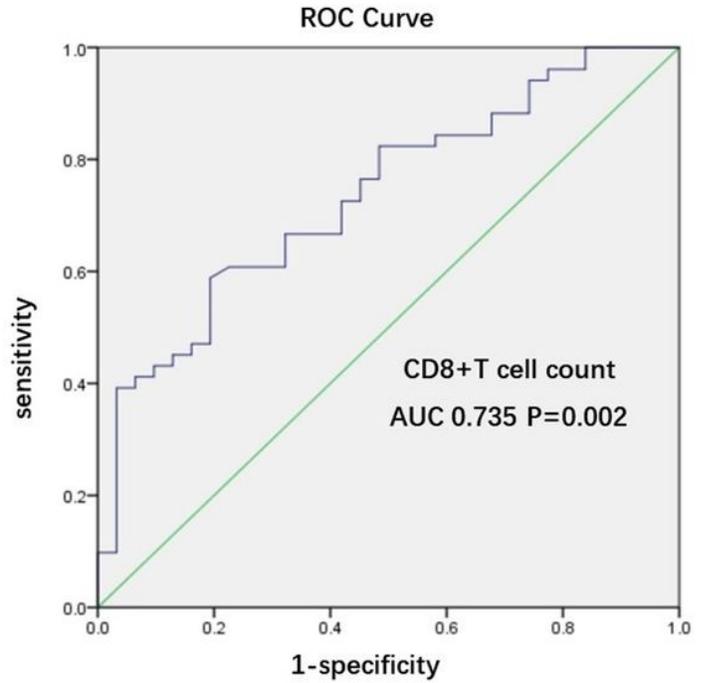
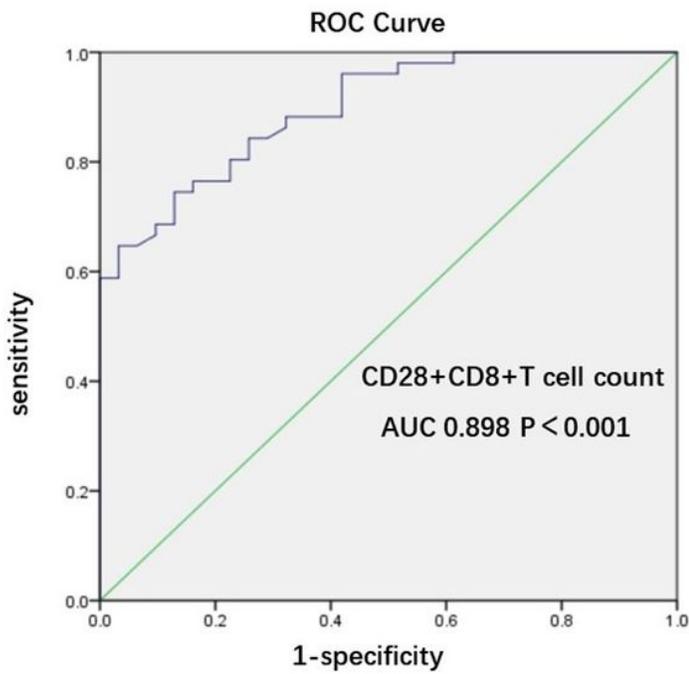
AUC area under the curve; CI confidence interval

## Figures



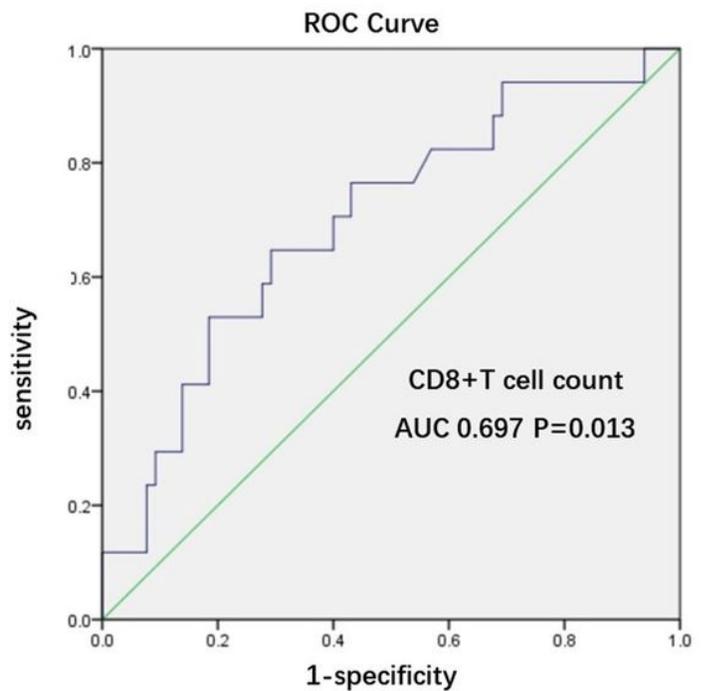
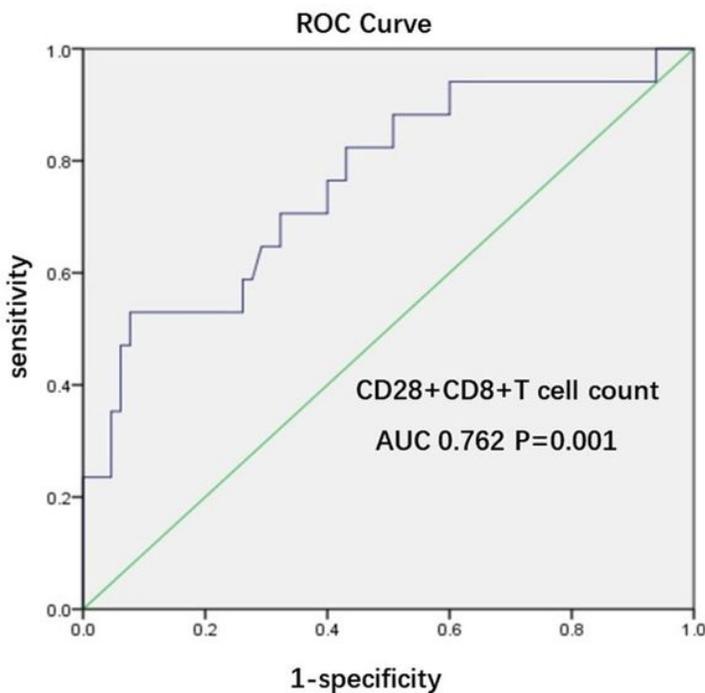
**Figure 1**

Flow chart of patient enrollment.



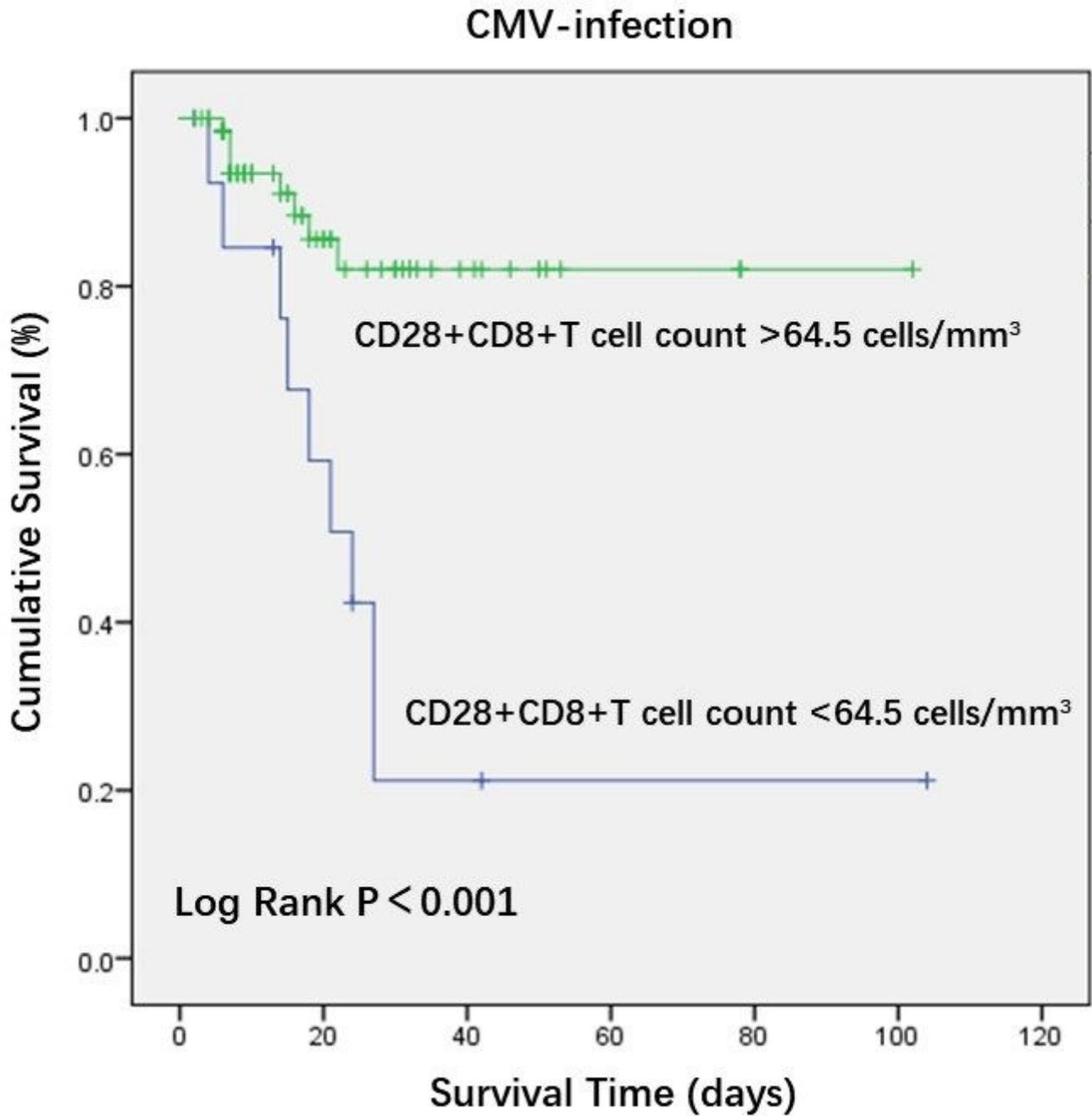
**Figure 2**

Receiver operating characteristic (ROC) curve analysis of parameters predicting cytomegalovirus-DNA negative conversion. AUC, area under the curve.



**Figure 3**

Receiver operating characteristic (ROC) curve analysis of parameters predicting 28-day mortality. AUC, area under the curve.



**Figure 4**

Kaplan–Meier analysis of survival probabilities in patients with cytomegalovirus infection. Survival measured according to CD28+CD8+ T-cell count <64.5 cells/mm<sup>3</sup> at intensive care unit admission. Survival time censored on day 28.