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

Guangxu Bai
Department of Critical Care Medicine, Peking Union Medical College Hospital, Peking Union Medical College and Chinese Academy of Medical Science

Hao Wang
Department of Clinical Laboratory, Peking Union Medical College Hospital, Peking Union Medical College, Chinese Academy of Medical Science

Na Cui (pumchcn@163.com)
Peking Union Medical College Hospital

Wei Cheng
Department of Critical Care Medicine, Peking Union Medical College Hospital, Peking Union Medical College and Chinese Academy of Medical Science,

Wen Han
Department of Critical Care Medicine, Peking Union Medical College Hospital, Peking Union Medical College and Chinese Academy of Medical Science

Jianwei Chen
Department of Critical Care Medicine, Peking Union Medical College Hospital, Peking Union Medical College and Chinese Academy of Medical Science

Research

Keywords: cytomegalovirus, T-lymphocyte subtyping, CMV-DNA negative conversion, sepsis

DOI: https://doi.org/10.21203/rs.3.rs-133310/v1

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 $CD28^+CD8^+$ and $CD8^+$ 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 $CD8^+$ and $CD28^+CD8^+$ T-cell counts were both independent risk factors for CMV-DNA negative conversion, with the latter having the better predictive ability. Lower $CD28^+CD8^+$ 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) $CD28^+CD8^+$ T cells/ml at ICU admission, respectively.

Conclusions: Sepsis patients with CMV infection had higher 28-day mortality than those without CMV infection. $CD28^+CD8^+$ 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, $CD28^+CD8^+$ 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 (identifier ChiCTR-ROC-17010750)

Introduction

According to Sepsis3.0[1], host immune imbalance is the core mechanism for lethal organ dysfunction in sepsis patients, and the main reason for susceptibility to multiple pathogens[2-3]. Cytomegalovirus (CMV) is the most common opportunistic virus in immunocompromised hosts, such as those with organ transplantation, HIV infection, and long-term chemotherapy[4-5], 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%[6-7]. 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[8].

CMV infection has an immune basis. It is closely related to a decrease in $CD4^+$ T-cell and natural killer (NK)-cell counts in patients with HIV infection or organ transplantation[9-10]. 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 (identifier ChiCTR-ROC-17010750).

Inclusion criteria were as follows: (1) age >18 years; (2) ICU stay ≥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 ≥2 as a result of the infection[1]. 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°C or hypothermia <36°C; leukocytosis (>12 000 cells/mm^3) or leukopenia (<4000 cells/mm^3); 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[12]. 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[13]. Skin and soft-tissue infection was diagnosed as soft-tissue infection accompanied by signs and symptoms of systemic toxicity[14]. Other infections included mediastinal infection and biliary or urinary tract infection, which have been described previously[15].

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 ≥500 copies/mL by real-time quantitative polymerase chain reaction (PCR), with or without clinical manifestations[16]. CMV negative conversion was defined as retesting of serum CMV DNA <500 copies/mL in patients with CMV infection during ICU/inpatient treatment[17]. 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) [18] and SOFA scores[19]. Life-sustaining treatments (need for mechanical ventilation, vasopressors or renal replacement therapy) for ≥24 h were recorded based on clinical evaluation and recent recommendations[20]. In accordance with the Third International Consensus Guidelines on the Management of Cytomegalovirus in Solid-Organ Transplantation[21] 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 uL) by real-time quantitative PCR using Therma-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[22]. 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 ($CD3^+$), $CD4^+$ T-cell subsets ($CD4^+CD3^+$ and $CD28^+CD4^+$), $CD8^+$ T-cell subsets ($CD8^+CD3^+$, $CD28^+CD8^+$), B cells ($CD19^+$) and NK cells ($CD3^-CD16^+CD56^+$).

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, $CD8^+$ T-cell count, $CD28^+$ T-cell count, and IgG level in CMV-infected sepsis patients had significant differences compared with those in the non-CMV-infected group. $CD8^+$ T-cell count is the sum of $CD28^+CD8^+$ and $CD28^-CD8^+$ T-cell counts, multicollinearity analysis was performed for all parameters with significant differences in the univariate analysis. VIF >5 was found for the $CD8^+$ T-cell count, suggesting collinearity with other variables. Thus, we performed $CD8^+$ and $CD28^+CD8^+$ T-cell counts in the multivariate logistic regression with other significantly different variables, respectively. The regression analysis showed that both $CD28^+CD8^+$ and $CD8^+$ 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 $CD8^+$ and $CD28^+$ T-cell counts differed significantly between these two groups (Tables 7 and 8). To avoid statistical error caused by multicollinearity, $CD8^+$ and $CD28^+CD8^+$ T-cell counts were similarly performed in logistic regression, respectively. Regression analysis suggested that $CD28^+CD8^+$ and $CD8^+$ T cell countss 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 $CD8^+$ T-cell count, $CD28^+CD8^+$ T-cell count had higher discriminatory power with an area under the curve of 0.898. A $CD28^+CD8^+$ T-cell count cutoff of 151 cells/mm^3 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. $CD8^+$ and $CD28^+$ 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, $CD28^+CD8^+$ and $CD8^+$ 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 $CD28^+CD8^+$ T-cell count may have been more accurate in predicting 28-day mortality after enrollment for CMV-infected sepsis patients. A lower $CD28^+CD8^+$ 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^3 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 $CD28^+CD8^+$ T-cell count <64.5 cells/mm^3 (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 $CD28^+CD8^+$ and $CD8^+$ 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. $CD28^+CD8^+$ T-cell count was the best predictor of CMV-DNA negative conversion potential in CMV-infected sepsis patients. A $CD28^+CD8^+$ T-cell count cutoff of 151 cells/mm^3 at ICU admission may predict the CMV-DNA negative conversion with a sensitivity of 74.5% and specificity 87.1%. A lower $CD28^+CD8^+$ T-cell count may be associated with a higher 28-day mortality. The sensitivity and specificity of a $CD28^+CD8^+$ T-cell count cutoff value of 64.5 cells/mm^3 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[23] 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 $CD8^+$ 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[24]. We found that NK-cell count, IgG and $CD28^+CD8^+$ 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.*[5], who found that $CD4^+$ T-cell count in HIV-infected patients could predict CMV infection. We did not find a significant difference in $CD4^+$ 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. $CD4^+$ T cells are the only specific target of HIV, and $CD4^+$ T-cell count of HIV-infected patients is closely related to susceptibility to opportunistic pathogens. Therefore, $CD4^+$ T-cell count could specifically represent the immune status of HIV-infected patients[25]. 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.*[26] showed that, although CMV infection was mainly controlled by $CD8^+$ and $CD4^+$ T cells, $CD8^+$ 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 $CD8^+$ T cells had faster CMV-DNA negative conversion.

The anti-CMV activity of $CD8^+$ T cells is closely related to their high sensitivity to CMV peptide fragments. It has been reported that approximately 30% of $CD8^+$ T cells in healthy adults are CMV reactive[27]. After the invasion of CMV, $CD8^+$ 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)[28], and co-stimulators such as CD28 and CD137[29], and cytokines such as interleukin (IL)-2 and IL-15[30-31]. These effector cells then circulate into the tissues and perform antiviral functions through perforin- and interferon-g-mediated mechanisms[32]. As an important co-stimulator of T cells, CD28 is involved in the activation and differentiation of $CD8^+$ T cells and is a typical surface marker that combines with others to classify functional subsets[33-34]. 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[35-36]. A study by Dolfi and colleague in 2016 has demonstrated that $CD28^+CD8^+$ T cells have good predictive value for the incidence of CMV infection in kidney transplantation patients[37]. Patients with lower $CD28^+CD8^+$ T-cell count have a higher incidence of CMV infection after kidney transplantation surgery, while for CMV-infected kidney transplantation patients, the increase of $CD28^+CD8^+$ 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[38]. Our findings are consistent with the above conclusions. First, by comparing the immune parameters of all sepsis patients, we found that $CD28^+CD8^+$ 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 $CD28^+CD8^+$ 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 $CD28^+CD8^+$ T-cell count was a better predictor of prognosis than $CD8^+$ T-cell count for CMV-infected sepsis patients. These results indicate that $CD28^+CD8^+$ 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.

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

the second and third stages of host resistance to CMV infection[24]. 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 $CD28^-CD8^+$ T-cell count between the groups. Another explanation for the lack of difference in $CD28^-CD8^+$ T-cell count between the groups in our study is age. Numerous studies[43-45] have indicated that $CD28^-CD8^+$ T cells are associated with immune senescence, and the proportion of $CD8^+$ 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 $CD8^+$ and $CD4^+$ 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 $CD28^+CD8^+$ T-cell count plays a potential role in prediction of CMV infection, CMV-DNA negative conversion and 28-day mortality in sepsis patients. Higher $CD28^+CD8^+$ 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.

Funding

The work was supported by Non-profit Central Research Institute Fund of Chinese Academy of Medical Sciences (No. 2019XK320040), National Natural Science Foundation of China (No. 81601657), Tibet Natural Science Foundation (No. XZ2019ZR-ZY12(Z)), and Wu Jieping Medical Foundation (No. 320.6750.18419).

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.

Acknowledgements

We are grateful to all the sepsis patients who participated in this study. We also acknowledge the staff at the ICU of Peking Union Medical College Hospital.

Availability of data and material

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

References

1. Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA 2016;315(8):801.
2. Rubio I, Osuchowski MF, Shankar-Hari M, et al. Current gaps in sepsis immunology: new opportunities for translational research. The Lancet Infectious Diseases 2019;19(12): e422-36.
3. Drewry AM, Samra N, Skrupky LP, et al. Persistent lymphopenia after diagnosis of sepsis predicts mortality. SHOCK 2014;42(5):383-91.
4. George MJ, Snydman DR, Werner BG, et al. The independent role of cytomegalovirus as a risk factor for invasive fungal disease in orthotopic liver transplant recipients. Boston Center for Liver Transplantation CMVIG-Study Group. Cytogam, MedImmune, Inc. Gaithersburg, Maryland. Am J Med Aug;1997 103(2):106–113.
5. Kaplan JE, Benson C, Holmes KK, et al. Guidelines for prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. MMWR Recomm Rep 2009;58(RR-4):1-207, E1-4.
6. Li X, Huang Y, Xu Z, Zhang R, Liu X, Li Y, Mao P. Cytomegalovirus infection and outcome in immunocompetent patients in the intensive care unit: a systematic review and meta-analysis. BMC INFECT DIS 2018;18(1):289.
7. Heininger A, Haeberle H, Fischer I, Beck R, Riessen R, Rohde F, et al. Cytomegalovirus reactivation and associated outcome of critically ill patients with severe sepsis. Crit Care. 2011;15: R77
8. Kalil AC, Florescu DF. Prevalence and mortality associated with cytomegalovirus infection in non-immunosuppressed patients in the intensive care unit. CRIT CARE MED 2009;37(8):2350-8.
9. Tay-Kearney ML, Enger C, Semba RD, Royal WR, Dunn JP, Jabs DA. T cell subsets and cytomegalovirus retinitis in human immunodeficiency virus-infected patients. J INFECT DIS 1997;176(3):790-4.

10. Fishman JA, Emery V, Freeman R, et al. Cytomegalovirus in transplantation-challenging the status quo. CLIN TRANSPLANT 2007;21(2):149-58.
11. Guidelines for the Management of Adults with Hospital-acquired, Ventilator-associated, and Healthcare-associated Pneumonia. AM J RESP CRIT CARE 2005;171(4):388-416.
12. Centers for Disease Control and Prevention. National Healthcare Safety Network (NHSN) patient safety component manual. https://www.cdc.gov/nhsn/pdfs/ pscmanual/pcsmanual_current.pdf (Accessed March 2017)
13. Mazuski JE, Solomkin JS. Intra-Abdominal Infections. The surgical clinics of North America 2009;2(89):421-37.
14. Stevens DL, Bisno AL, Chambers HF, et al. Practice Guidelines for the Diagnosis and Management of Skin and Soft-Tissue Infections. CLIN INFECT DIS 2005(41):1373-406.
15. Cheng W, Wang H, Zhang J, et al. Lymphocyte subset counts as diagnostic and prognostic markers for carbapenem-resistant Enterobacteriaceae (CRE) infection in critically ill patients. INT J INFECT DIS 2020; 96:315-22.
16. Qin L, Qiu Z, Hsieh E, et al. Association between lymphocyte subsets and cytomegalovirus infection status among patients with systemic lupus erythematosus: A pilot study. Medicine (Baltimore) 2019;98(39): e16997.
17. Dioverti MV, Razonable RR. Clinical utility of cytomegalovirus viral load in solid organ transplant recipients. CURR OPIN INFECT DIS 2015;28(4):317-22.
18. Knaus WA, Draper EA, Wagner DP, Zimmerman JE: APACHE II: a severity of disease classification system. Crit Care Med 1985, 13:818–829.
19. Vincent JL, Moreno R, Takala J, et al, on behalf of the Working Group on Sepsis related Problems of the European Society of Intensive Care Medicine: The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. Intensive Care Med 1996, 22:707–710.
20. Rhodes A, Evans LE, Alhazzani W, et al. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. CRIT CARE MED 2017;45(3):486-552.
21. Kotton CN, Kumar D, Caliendo AM, et al. The Third International Consensus Guidelines on the Management of Cytomegalovirus in Solid-organ Transplantation. TRANSPLANTATION 2018;102(6):900-31.
22. Cui N, Wang H, Long Y, et al. CD8(+) T-cell counts: an early predictor of risk and mortality in critically ill immunocompromised patients with invasive pulmonary aspergillosis. CRIT CARE 2013;17(4): R157.
23. Podlech J, Ebert S, Becker M, et al. Mast cells: innate attractors recruiting protective CD8 T cells to sites of cytomegalovirus infection. Med Microbiol Immunol. 2015
24. Picarda G, Benedict CA. Cytomegalovirus: Shape-Shifting the Immune System. The Journal of Immunology 2018;200(12):3881-9.

25. Fauci AS, Pantaleo G, Stanley S, Weissman D. Immunopathogenic Mechanisms of HIV Infection. ANN INTERN MED 1996;7(124):654-63.
26. Radha R, Jordan S, Puliyanda D, et al. Cellular immune responses to cytomegalovirus in renal transplant recipients. AM J TRANSPLANT 2005;5(1):110-7.
27. Sylwester AW, Mitchell BL, Edgar JB, et al. Broadly targeted human cytomegalovirus specific CD4+ and CD8+ T cells dominate the memory compartments of exposed subjects. J EXP MED 2005;202(5):673-85.
28. Slezak SL, Bettinotti M, Selleri S, et al. CMV pp65 and IE-1 T cell epitopes recognized by healthy subjects. J TRANSL MED 2007: 5:17.
29. Waller EC, McKinney N, Hicks R, et al. Differential costimulation through CD137 (4-1BB) restores proliferation of human virus-specific "effector memory" (CD28(-) CD45RA(HI)) CD8(+) T cells. BLOOD 2007;110(13):4360-6.
30. Malek TR, Castro I. Interleukin-2 receptor signaling: at the interface between tolerance and immunity. IMMUNITY 2010;33(2):153-65.
31. Patterson J, Jesser R, Weinberg A. Distinctive in vitro effects of T-cell growth cytokines on cytomegalovirus-stimulated T-cell responses of HIV-infected HAART recipients. VIROLOGY 2008;378(1):48-57.
32. Sumaria N, van Dommelen SL, Andoniou CE, Smyth MJ, Scalzo AA, Degli-Esposti MA. The roles of interferon-gamma and perforin in antiviral immunity in mice that differ in genetically determined NK-cell-mediated antiviral activity. IMMUNOL CELL BIOL 2009;87(7):559-66.
33. Speiser DE, Migliaccio M, Pittet MJ, et al. Human CD8(+) T cells expressing HLA-DR and CD28 show telomerase activity and are distinct from cytolytic effector T cells. EUR J IMMUNOL 2001;31(2):459-66.
34. Rudd CE, Taylor A, Schneider H. CD28 and CTLA-4 coreceptor expression and signal transduction. IMMUNOL REV 2009;229(1):12-26.
35. Fang M, Sigal LJ. Direct CD28 Costimulation Is Required for CD8+ T Cell-Mediated Resistance to an Acute Viral Disease in a Natural Host. The Journal of Immunology 2006;177(11):8027-36.
36. Dolfi DV, Duttagupta PA, Boesteanu AC, et al. Dendritic cells and CD28 costimulation are required to sustain virus-specific CD8+T cell responses during the effector phase in vivo. J IMMUNOL 2011;186(8):4599-608.
37. Zielinski M, Tarasewicz A, Zielinska H, et al. CD28 positive, cytomegalovirus specific cytotoxic T lymphocytes as a novel biomarker associated with cytomegalovirus viremia in kidney allorecipients. J CLIN VIROL 2016; 83:17-25.
38. Monserrat J, de Pablo R, Reyes E, et al. Clinical relevance of the severe abnormalities of the T cell compartment in septic shock patients. Crit Care. 2009; 13(1): R26
39. Moss P, Khan N. CD8(+) T-cell immunity to cytomegalovirus. HUM IMMUNOL 2004;65(5):456-64.

40. Weekes MP, Carmichael AJ, Wills MR, et al. Human CD28-CD8+ T cells contain greatly expanded functional virus-specific memory CTL clones. J IMMUNOL 1999;162(12):7569-77.
41. Chen X, Liu Q, Xiang AP. CD8+CD28- T cells: not only age-related cells but a subset of regulatory T cells. Cellular & Molecular Immunology 2018(15):734-6.
42. Tovar-Salazar A, Weinberg A. Understanding the mechanism of action of cytomegalovirus-induced regulatory T cells. VIROLOGY 2020; 547:1-6.
43. Weng N, Akbar AN, Goronzy J. CD28-T cells: their role in the age-associated decline of immune function. TRENDS IMMUNOL 2009;30(7):306-12.
44. Ventura MT, Casciaro M, Gangemi S, Buquicchio R. Immunosenescence in aging: between immune cells depletion and cytokines up-regulation. Clin Mol Allergy 2017;15:21.
45. Pangrazzi L, Weinberger B. T cells, aging and senescence. EXP GERONTOL 2020;134:110887.

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
Underlying disease (n (%))				
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
Life-sustaining treatments (n (%))				
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
Sites of infection (n (%))				
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
Multiple site infection (n (%))				
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
Pathogens (n (%))				
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
Drug therapy (n (%))				
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
Biochemical parameters at ICU admission				
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
Infection marker at ICU admission				
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
Outcome				
ICU durations (days)	10(13)	16.5(22)	9(11)	0.000
Hospital durations (days)	20(23)	23(23)	19(23)	0.114
ICU motality (n(%))	71(11.9)	17(20.7)	54(10.4)	0.007
Hospital motality (n(%))	84(14)	19 (23.2)	65 (12.6)	0.010
28 days motality (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[1]
WBC (10^9/L)	11.79 (8.9)	10.45 (8.31)	12.07 (9)	0.112
NG (10^9/L)	9.95 (8.27)	8.37 (7.50)	10.04 (8.63)	0.154
NK (cells/mm^3)	74 (85)	59.5 (54)	76 (90)	0.002
B cells	106 (119)	96 (87)	106 (123)	0.071
Lymphocyte (cells/mm^3)	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
Complement factor (g/L)				
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
Immunoglobulin (g/L)				
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 value1
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 value1
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 (10^9/L)	10.45 (8.2)	9.90 (8.08)	10.87 (7.62)	0.257
NG (10^9/L)	8.37 (7.44)	8.12 (7.91)	8.67 (7.56)	0.202
NK (cells/mm^3)	60.5 (57)	59 (64)	61 (64)	0.852
B cells (cells/mm^3)	101 (91.25)	93 (73)	113 (101)	0.579
Lymphocyte (cells/mm^3)	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
Complement factor (g/L)				
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
Immunoglobulin (g/L)				
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 $CD8^+$ T-cell count distinguishing CMV-DNA negative conversion

Parameters	OR	95%CI	P value1
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 $CD28^+CD8^+$ T-cell count distinguishing CMV-DNA negative conversion

Parameters	OR	95%CI	P value1
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^3)	358	73.5	0.627-0.844	0.000
CD28+CD8+T (cells/mm^3)	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 (10^9/L)	10.45 (8.2)	10.31 (8.03)	10.59 (11.47)	0.194
NG (10^9/L)	8.37 (7.44)	8.32 (6.99)	8.41 (10.49)	0.255
NK (cells/mm3)	60.5 (57)	59 (60)	61 (49)	0.895
B cells	101 (91.25)	97 (85)	95 (149)	0.864
Lymphocyte (cells/mm3)	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 $CD28^+CD8^+$ 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 $CD8^+$ 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/mm3□	266	69.7	0.555-0.839	0.013
CD28+CD8+T□cells/mm3□	64.5	76.2	0.627-0.897	0.001

AUC area under the curve; *CI* confidence interval

Figures

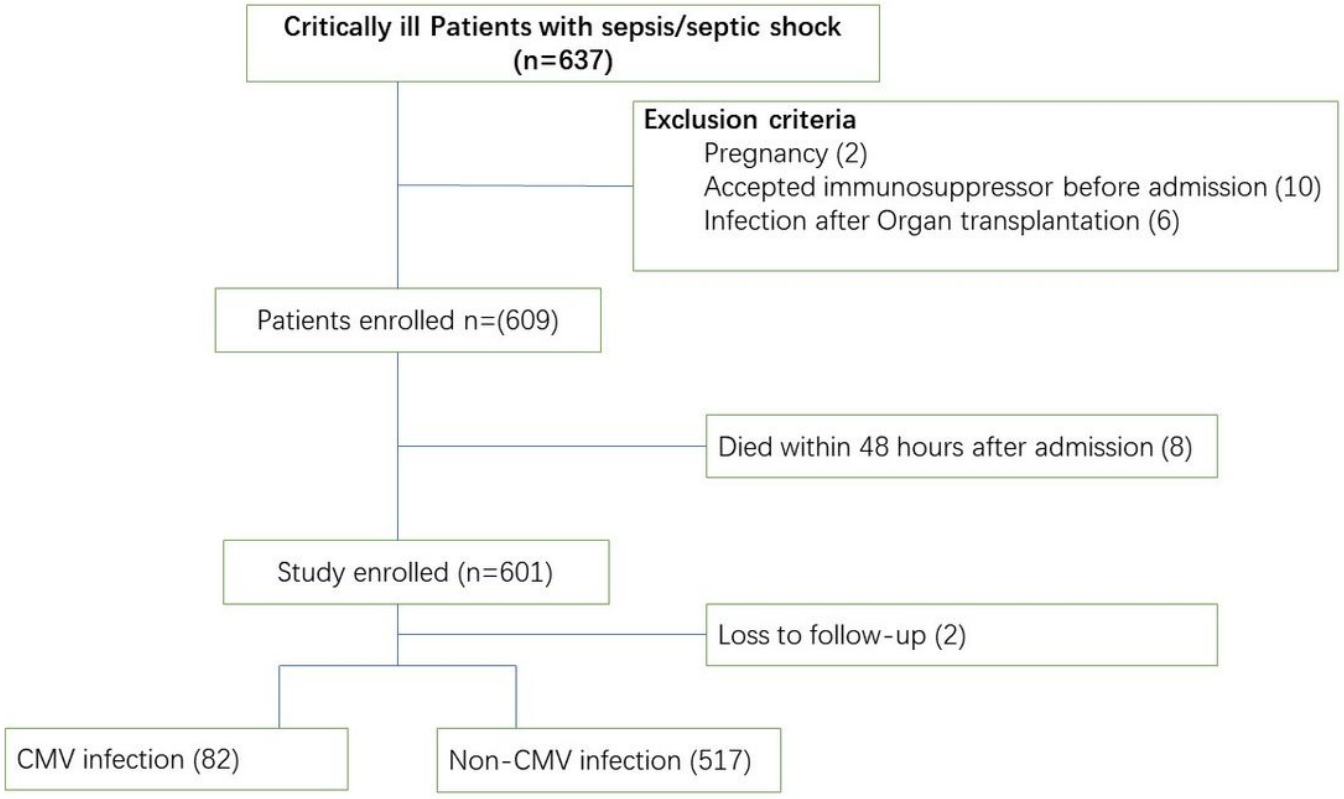

Figure 1

Flow chart of patient enrollment.

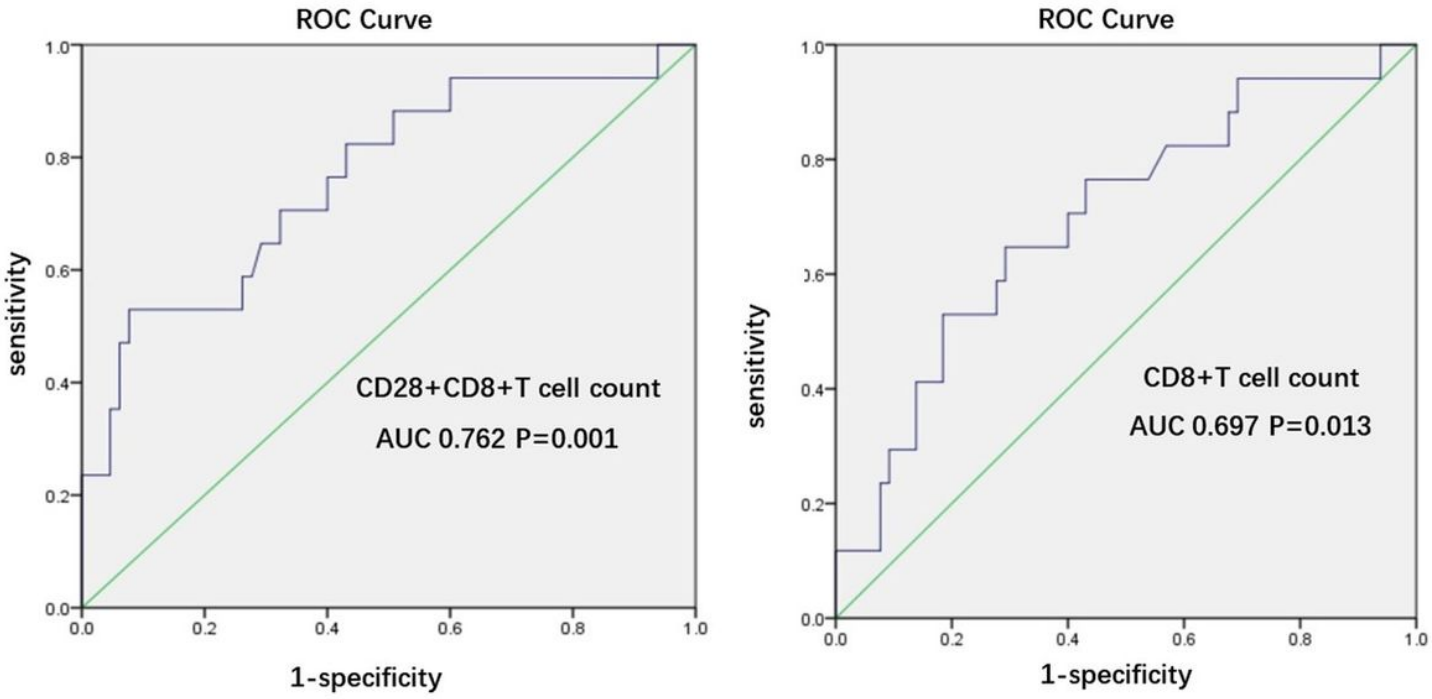

Figure 3

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