T cell subset counts in peripheral blood can be used as discriminatory biomarkers for diagnosis and severity prediction of COVID-19

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Summary: Our results demonstrated that significantly decreased T cell subset counts were related to the severity and prognosis of COVID-19. Consequently, the counts of CD8+T and CD4+T cells can be used as diagnostic markers of COVID-19 and predictors of disease severity.
Abstract

This study evaluated the significance of lymphocyte subsets detection in peripheral blood in the diagnosis and prognosis of coronavirus disease 2019 (COVID-19). Our results revealed that CD3+T, CD4+T, CD8+T cells and NK cells were significantly decreased in COVID-19 patients. COVID-19 patients had a relatively slight decrease in CD4+T cells but a severe decrease of CD8+T cells. The significantly elevated CD4/CD8 ratio was observed in COVID-19 patients. T cell subset counts were related to the severity and prognosis of COVID-19. The counts of CD8+T and CD4+T cells can be used as diagnostic markers of COVID-19 and predictors of disease severity.

Keywords: SARS-CoV2; lymphocyte subsets; diagnosis; prognosis
Introduction

Coronavirus disease 2019 (COVID-19) is viral pneumonia that affects humans. It is caused by a novel coronavirus that the International Committee on Taxonomy of Viruses identified as severe acute respiratory syndrome coronavirus 2 (SARS-CoV2). The infection induces groups of severe respiratory illnesses that are similar to severe acute respiratory syndrome coronavirus and are associated with intensive care unit (ICU) admission [1]. Recent studies have reported that T lymphocyte, as well as the counts of inflammatory cytokines in the peripheral blood, are correlated with the severity of COVID-19 [2, 3]. Yet, the significance of lymphocyte subsets in peripheral blood in the diagnosis and prognosis of COVID-19 still needs to be elucidated. In this study, we investigated the counts of lymphocyte subsets in COVID-19 patients and evaluated the significance of detection of lymphocyte subsets in peripheral blood in the diagnosis, disease assessment, and prognosis of COVID-19.

Materials and Methods

Patient variables

A total of 103 COVID-19 patients (58 males and 45 females) with a median age of 46 years (17~88) treated at the First Affiliated Hospital of Nanchang University between January 30, 2020 and February 16, 2020, were enrolled in the study. Among them, 86 (47 males and 39 females) were mild-to-moderate patients with a median age of 44 years (17~83) and 17 (11 males and 6 females) were severe patients treated in ICU with a median age of 62 years (41~88). All patients were confirmed to have SARS-CoV2 infection by virus nucleic acid test. Thirteen healthy controls (HCs) who did not have any infectious disease and were unrelated
to the COVID-19 patients were enrolled in the study as the control group.

The study was approved by the Ethics Committee of the First Affiliated Hospital of Nanchang University and was performed in compliance with the Declaration of Helsinki. Informed consent forms were obtained from all participants.

**Lymphocyte subsets and Treg cells detection**

The EDTA-anticoagulated peripheral blood samples were collected from all subjects. Lymphocyte subsets were detected and counted by Cytomics FC 500 flow cytometer (Beckman Coulter, Brea, CA, USA), and the subsets were characterized by corresponding phenotypes of CD antigens. The following antibodies (Beckman Coulter, USA) were used: FITC-conjugated anti-CD4, PE-conjugated anti-CD19, ECD-conjugated anti-CD3, PC5-conjugated anti-CD8 and PC7-conjugated anti-CD45 (Beckman Coulter, Brea, CA, USA) were used for lymphocyte subsets detection. FITC-conjugated anti-CD4, PE-conjugated anti-CD127, and PC5-conjugated anti-CD25 were used for Treg cells detection. Tests were performed according to the product manual.

**Statistical analyses**

Statistical analysis was carried out with GraphPad Prism 5.0 and SPSS 17.0. Continuous variables were expressed as mean ± standard deviation or median (quartile) [M (P25, P75)] depending on whether data conformed to normal distribution. Student t-test or Mann-Whitney test were used for statistical analysis according to the data distribution. The receiver operating characteristic (ROC) curve was used to assess the diagnostic value of lymphocyte subsets. A
value of \( P < 0.05 \) indicated statistical significance.

**Results**

**Lymphocyte subsets in peripheral blood of COVID-19 patients**

Significant decreases were observed in the counts of CD3+T, CD4+T, CD8+T, NK cells, as well as increases in the ratio of CD4/CD8 in COVID-19 patients compared to HCs (all \( P<0.05 \)). There was no statistical difference detected in B cells between the two groups (\( P>0.05 \))(Table S1).

**Lymphocyte subsets in peripheral blood between mild-to-moderate patients and severe COVID-19 patients**

Severe COVID-19 patients showed significant decreases in lymphocyte subsets counts compared to mild-to-moderate patients, especially in CD3+T, CD4+T, and CD8+T cells (all \( P<0.05 \)). There was no significant difference in the percentage of Treg cells between mild-to-moderate and severe COVID-19 patients (\( P>0.05 \))(Table S2).

**Changes of the counts of lymphocyte subsets in COVID-19 patients during follow-up**

23 newly diagnosed COVID-19 patients were followed up within 2 weeks. After the follow-up, the counts of CD3+T, CD4+T and CD8+T cells dramatically recovered in most patients whose virus nucleic acid test turned negative (Figure1a-c), but showed no significant difference in patients with a persistent positive nucleic acid test (Figure S1a-c). The counts of NK and B cells in 23 newly diagnosed COVID-19 patients showed no significant change (\( P>0.05 \)) during the follow-up (Figure S2a-d).
T cell subset counts in peripheral blood can be used as discriminatory biomarkers for diagnosis and disease severity prediction of COVID-19

Based on the above results, we selected CD4+T and CD8+T cells as candidate diagnostic markers in the diagnosis of COVID-19 and prediction of disease severity. ROC curves indicated that the counts of CD4+T and CD8+T in peripheral blood could differentiate between COVID-19 patients and HCs. The higher area under the curve (AUC) was CD8+T [AUC =0.8876; 95% confidence interval (CI), 0.8197 -0.9555; P<0.0001; sensitivity =100%, specificity =68.93%; Figure 2a], followed by CD4+T (AUC=0.7573; 95% CI, 0.6605-0.8541; P=0.0026; sensitivity=100%; specificity =57.28%; Figure 2a). The risk value of CD8+T and CD4+T used for distinguishing between COVID-19 patients and HCs were 285.5/ul and 386.0/ul. The combined AUC of CD8+T and CD4+T (AUC=0.9029; 95% CI, 0.8397 to 0.9661; P<0.0001; sensitivity =92.31%; specificity =79.61%; Figure 2b) was higher than individual CD8+T or CD4+T AUC value. ROC curves also indicated that the counts of CD4+T and CD8+T in peripheral blood could differentiate between severe patients and mild-to-moderate patients. A little higher area under the curve (AUC) was CD4+T [AUC =0.8666; 95% confidence interval (CI), 0.7746-0.9587; P<0.0001; sensitivity =88.24%, specificity =76.74%; Figure 2c], followed by CD8+T (AUC=0.8618; 95% CI, 0.7744-0.9492; P<0.0001; sensitivity=82.35%; specificity =82.56%; Figure 2c). The risk value of CD8+T and CD4+T used for distinguishing between severe patients and mild-to-moderate patients were 103.5/ul and 238.5/ul. The combined AUC of CD8+T and CD4+T (AUC=0.8810; 95% CI, 0.8009 -0.9611; P<0.0001; sensitivity =88.24%; specificity =80.23%; Figure 2d) was a little higher than individual CD8+T or CD4+T AUC value.
Discussion

Lymphocyte subsets are important factors for preserving immune function. Lymphocyte subsets in peripheral blood of patients with infectious diseases tend to abnormally change. Previous studies have reported that a significant decrease in both CD4+T and CD8+T cells was observed in patients with SARS compared to HCs. CD4+T cells are more severely damaged by the SARS virus than CD8+T cells [4, 5]. Our results revealed significant decreases in the counts of CD3+T, CD4+T, CD8+T, NK cells, especially CD3+T, CD8+T, and NK cell, as well as increases in the CD4/CD8 ratio in COVID-19 patients compared to those in the HCs. This implied that T lymphopenia, and in a particular decrease of CD8+T, was more common among COVID-19 patients than CD4+T, which differs from SARS-CoV infection. It has also been reported that the counts of T lymphocytes are related to the severity of SARS patients [4]. We found that severe patients admitted in ICU showed significant decreases in a count of lymphocyte subset compared to mild-to-moderate patients, especially CD3+T, CD4+T, and CD8+T cells. This indicated that T lymphocytes were more suppressed in severe patients compared to B cells and NK cells, which is consistent with recent studies [2, 3]. Researchers reported on two causes of T lymphopenia in SARS: sequestration into the lung of β-chemokine-recruited lymphocytes and IFN-γ-induced apoptosis [6]. Nevertheless, the underlying mechanism of the decrease of T lymphocytes in COVID-19 patients remains unclear and needs to be elucidated by further studies.

Treg cells are of great importance for the regulation of the magnitude of the immune response to infection. The function of Treg cells can protect the host versus excessive inflammation and
tissue damage [7]. However, they can also be abnormally induced by some viruses maintaining viral infection, such as hepatitis C virus, hepatitis B virus, and Epstein-Barr virus [8-10]. Our study revealed that there was no significant difference in the percentage of Treg cells between mild-to-moderate patients and severe patients. We speculated that Treg cells might not have a critical role in SARS-CoV2 infection, and multicenter researches are needed to confirm our hypothesis.

According to the diagnosis and treatment of pneumonia caused by a new coronavirus infection (trial version 7) [11], two negative new coronavirus nucleic acid tests were one of the conditions for releasing patients from isolation, which to some extent indicated that the patient was recovering from an infection. We found that the counts of CD3+T, CD4+T and CD8+T cells dramatically recovered in most follow-up patients whose virus nucleic acid test turned negative, but showed no significant difference for patients with the persistent positive nucleic acid test. It has been confirmed that T-cell immune response has an important role in recovery from SARS-CoV2 infection. Liu et al [3] also found that the decrease of T cells in the severe patient group reached its peak within the first week during the disease course, after which T cell numbers gradually increased from the second week and recovered to a count that was comparable count to the mild patient group in the third week; all the severe patients survived the disease. These results implied that the restoration of T lymphocytes was a favorable outcome.

Early diagnosis of COVID-19 patients and the identification of severe patients may facilitate the provision of appropriate supportive care. Based on our results, we selected CD4+T and
CD8+T as candidate diagnostic markers in the diagnosis of COVID-19 patients and the prediction of severe patients. Comparison between COVID-19 patients and HCs revealed that CD4+T had an AUC value of 0.7573, CD8+T had an AUC value of 0.8876, and the combination of CD4+T and CD8+T had an AUC value of 0.9029, thus indicating that the counts of CD4+T and CD8+T in peripheral blood have potential diagnostic value for COVID-19. The risk values of CD8+T and CD4+T used for distinguishing between COVID-19 patients and HCs were 285.5/ul and 386.0/ul, respectively. Comparison between severe patients and mild-to-moderate patients revealed that CD4+T had an AUC value of 0.8666, CD8+T had an AUC value of 0.8618, and the combination of CD4+T and CD8+T had an AUC value of 0.8810, thus demonstrating that CD4+T and CD8+T also distinguished severe patients from mild-to-moderate patients. The risk value of CD8+T and CD4+T used for distinguishing between severe patients and mild-to-moderate patients were 103.5/ul and 238.5/ul, respectively. Recent researches have reported other prognostic factors such as neutrophil-to-lymphocyte ratio (NLR) and neutrophil-to-CD8+T cell ratio (N8R), which had even better performance than NLR in the ROC curve analysis [3, 12]. Their kinetic analysis revealed that CD8+ T cells are the major lymphocyte subset, which decreases in cell numbers during COVID-19 [3], which is consistent with our findings.

In summary, our results demonstrated that CD3+T cells, CD4+T cells, CD8+T cells, and NK cells were significantly decreased in COVID-19 patients and related to the severity and prognosis of COVID-19. Consequently, the counts of CD8+T and CD4+T cells can be used as diagnostic markers of COVID-19 and predictors of disease severity. To the best of our
knowledge, this is the first work that described Treg cells in COVID-19 patients; still, we found no significant difference in Treg cells percentage between mild-to-moderate patients and severe patients. This suggests that Treg cells may not have a critical role in SARS-CoV2 infection. Of note, the sample size in our study was relatively small, and multicenter researches are needed to confirm our hypothesis. To conclude, we found the risk value of CD8+T and CD4+T cells for the diagnosis of COVID-19 cases and the prediction of severe patients. Immunological characteristics of the lymphocyte subsets are important for exploring the mechanisms underlying SARS-CoV2 infection. Large-scale multicenter clinical studies are needed to elucidate lymphocyte subsets in the immunological mechanisms of COVID-19.

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Author contributions

MJ and YG performed the experiments and drafted the manuscript. QL, ZKH, RZ, SYL discussed the advice from reviewers and revised it together. APL, JML, LGW analyzed and interpreted the data and approved the final manuscript. All of the authors have read and approved the manuscript.

Conflict of interest
We declare no conflicts of interest.

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Figure legends

Figure 1. The dynamic changes of CD3+T (a), CD4+T (b) and CD8+T (c) cells in COVID-19 patients whose viral nucleic acid test turned negative within the 2-week follow-up.

Figure 2. Receiver operating characteristic analysis of CD4+T and CD8+T in peripheral blood from COVID-19 patients and HCs. (a) The higher AUC was identified for CD8+T, followed by CD4+T. (b) The combined AUC of CD8+T and CD4+T was 0.9029. Receiver operating characteristic analysis of CD4+T and CD8+T in peripheral blood from severe patients and mild-to-moderate patients (c) The higher AUC was identified for CD4+T, followed by CD8+T. (d) The combined AUC of CD8+T and CD4+T was 0.8810.
Figure 1

(a) P=0.0074  
(b) P=0.0136  
(c) P=0.0089
Figure 2

(a) ROC Curve for COVID-19 vs Controls

(b) ROC Curve for Combined CD4 and CD8

(c) ROC Curve for Mid-to-Moderate Patients vs Severe Patients

(d) ROC Curve for Combined CD4 and CD8