The Journal of Infectious Diseases

BRIEF REPORT

T-Cell Subset Counts in Peripheral Blood Can Be Used as Discriminatory Biomarkers for Diagnosis and Severity Prediction of Coronavirus Disease 2019

Mei Jiang,[1,a] Yang Guo,[1,a] Qing Luo,[1] ZiKun Huang,[1] Rui Zhao,[1] ShuYuan Liu,[1] AiPing Le,[2,b] JunMing Li,[1,b] and LaGen Wan[1]

[1]Department of Clinical Laboratory, First Affiliated Hospital of Nanchang University, Nanchang, Jiangxi, China, and [2]Department of Transfusion, First Affiliated Hospital of Nanchang University, Nanchang, Jiangxi, China

This study evaluated the significance of lymphocyte subset detection in peripheral blood in the diagnosis and prognosis of coronavirus disease 2019 (COVID-19). Our results revealed that $CD3^+$ T cells, $CD4^+$ T cells, $CD8^+$ T cells, and natural killer cells were significantly decreased in patients with COVID-19. These patients had a relatively slight decrease in $CD4^+$ T cells but a severe decrease in $CD8^+$ 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, suggesting that 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.

Coronavirus disease 2019 (COVID-19) is a 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-CoV-2). The infection induces groups of severe respiratory illnesses that are similar to those observed in severe acute respiratory syndrome coronavirus (SARS) and are associated with intensive care unit (ICU) admission [1]. Recent studies have reported that T lymphocytes, 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 cases 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 patients with COVID-19 (58 males and 45 females) with a median age of 46 years (17–88 years), treated at the First Affiliated Hospital of Nanchang University between 30 January 2020 and 16 February 2020, were enrolled in the study. Among them, 86 (47 males and 39 females) were patients with mild-to-moderate illness, with a median age of 44 years (17–83 years), and 17 (11 males and 6 females) were patients with severe illness treated in ICU, with a median age of 62 years (41–88 years). All patients were confirmed to have SARS-CoV-2 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 Regulatory T-Cell Detection

Ethylenediaminetetraacetic acid–anticoagulated peripheral blood samples were collected from all subjects. Lymphocyte subsets were detected and counted by Cytomics FC 500 flow cytometer (Beckman Coulter, Brea, California), and the subsets were characterized by corresponding phenotypes of CD antigens. The following antibodies (Beckman Coulter) were used: fluorescein isothiocyanate (FITC)–conjugated anti-CD4, R-phycoerythrin (PE)–conjugated anti-CD19, PE-texas red (ECD)–conjugated anti-CD3, PE-Cy5 (PC5)–conjugated anti-CD8, and PE-Cy7 (PC7)-conjugated anti-CD45 (Beckman Coulter) were used for lymphocyte subset detection. FITC-conjugated anti-CD4, PE-conjugated anti-CD127, and PC5-conjugated anti-CD25 were used for regulatory T-cell (Treg) 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 (first quartile, third quartile [Q1, Q3]) 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 $P < .05$ indicated statistical significance.

Received 17 March 2020; editorial decision 1 May 2020; accepted 6 May 2020; published online May 7, 2020.

[a]M. J. and Y. G. contributed equally to this work.

[b]A. L. and J. L. contributed equally and share the co-corresponding authorship.

Correspondence: L. Wan, MBBS, Department of Clinical Laboratory, First Affiliated Hospital of Nanchang University, 17 Yongwai Zheng Jie, Nanchang, Jiangxi 330006, PR China (wlgme196412@126.com).

The Journal of Infectious Diseases® 2020;222:198–202

DOI: 10.1093/infdis/jiaa252

RESULTS

Lymphocyte Subsets in Peripheral Blood of Patients With COVID-19

Significant decreases were observed in the counts of $CD3^+$, $CD4^+$, and $CD8^+$ T cells and natural killer (NK) cells, as well as increases in the ratio of CD4/CD8 in COVID-19 patients compared to HCs (all $P < .05$). There was no statistical difference detected in B cells between the 2 groups ($P > .05$) (Supplementary Table 1).

Lymphocyte Subsets in Peripheral Blood Between Patients With Mild-to-Moderate Versus Severe COVID-19

Patients with severe COVID-19 showed significant decreases in lymphocyte subsets counts compared to those with mild-to-moderate illness, especially in $CD3^+$, $CD4^+$, and $CD8^+$ T cells (all $P < .05$). There was no significant difference in the percentage of Treg cells between mild-to-moderate and severe COVID-19 cases ($P > .05$) (Supplementary Table 2).

Changes of the Counts of Lymphocyte Subsets in Patients With COVID-19 During Follow-up

Twenty-three newly diagnosed COVID-19 patients were followed up within 2 weeks. After the follow-up, the counts of $CD3^+$, $CD4^+$, and $CD8^+$ cells dramatically recovered in most patients whose virus nucleic acid test turned negative (Figure 1), but showed no significant difference in patients with a persistent positive nucleic acid test (Supplementary Figure 1). The counts of NK and B cells in the 23 newly diagnosed COVID-19 patients showed no significant change ($P > .05$) during the follow-up (Supplementary Figure 2).

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 $CD4^+$ 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 $CD4^+$ and $CD8^+$ T cells in peripheral blood could differentiate between COVID-19 patients and HCs. The higher area under the curve (AUC) was for $CD8^+$ T cells (AUC = 0.8876 [95% confidence interval {CI}, .8197–.9555]; $P < .0001$; sensitivity = 100%, specificity = 68.93%; Figure 2A), followed by $CD4^+$ T cells (AUC = 0.7573 [95% CI, .6605–.8541]; $P = .0026$; sensitivity = 100%; specificity = 57.28%; Figure 2A). The risk value of $CD8^+$ and $CD4^+$ T cells used for distinguishing between COVID-19 patients and HCs was 285.5/μL and 386.0/μL, respectively. The combined AUC of $CD8^+$ and $CD4^+$ T cells (AUC = 0.9029 [95% CI, .8009–.9611]; $P < .0001$; sensitivity = 92.31%; specificity = 79.61%; Figure 2B) was higher than individual $CD8^+$ or $CD4^+$ AUC values. ROC curves also indicated that the counts of $CD4^+$ and $CD8^+$ T cells in peripheral blood could differentiate between patients with severe vs mild-to-moderate illness. A little higher on the AUC was $CD4^+$ T cells (AUC = 0.8666 [95% CI, .7746–.9587]; $P < .0001$; sensitivity = 88.24%, specificity = 76.74%; Figure 2C), followed by $CD8^+$ T cells (AUC = 0.8618 [95% CI, .7744–.9492]; $P < .0001$; sensitivity = 82.35%; specificity = 82.56%; Figure 2C). The risk value of $CD8^+$ and $CD4^+$ T cells used for distinguishing between severe and mild-to-moderate cases was 103.5/μL and 238.5/μL, respectively. The combined AUC of $CD8^+$ T and $CD4^+$ T (AUC = 0.8810 [95% CI, .8009 -.9611]; $P < .0001$; sensitivity = 88.24%; specificity = 80.23%; Figure 2D) was a little higher than individual $CD8^+$ or $CD4^+$ 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 $CD3^+$, $CD4^+$, and $CD8^+$ T cells and NK cells, especially $CD3^+$, $CD8^+$, and NK cells, 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 particular a decrease of $CD8^+$ T cells, was more common among COVID-19 patients than $CD4^+$ T cells, which differs from SARS infection. It has also been reported

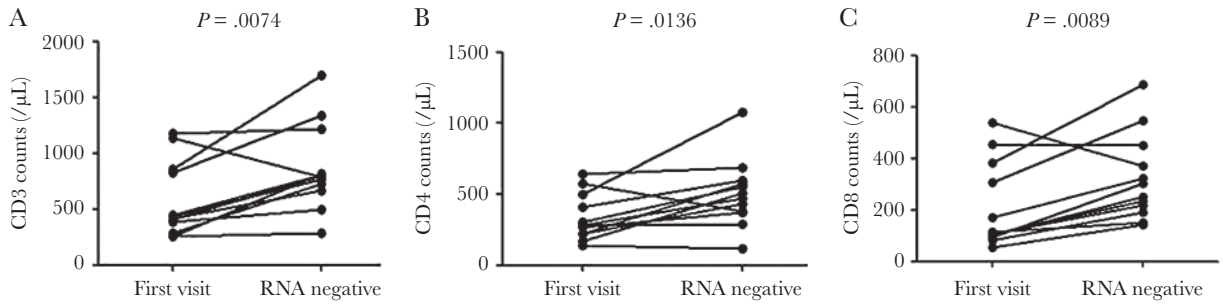

Figure 1. The dynamic changes of $CD3^+$ T cells (*A*), $CD4^+$ T cells (*B*), and $CD8^+$ T cells (*C*) in patients with coronavirus disease 2019 whose viral nucleic acid test turned negative within the 2-week follow-up.

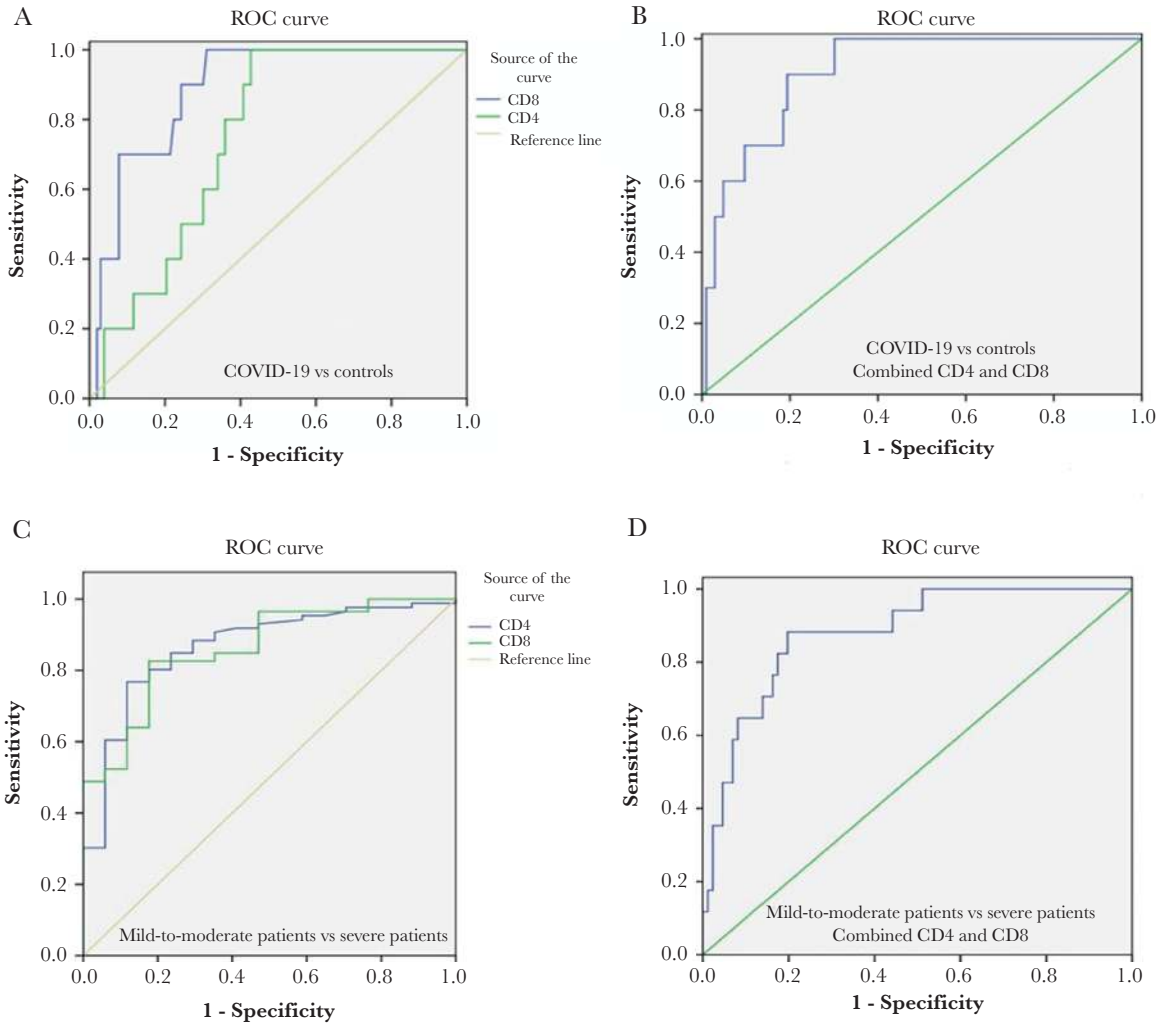

Figure 2. Receiver operating characteristic analysis of $CD4^+$ and $CD8^+$ T cells in peripheral blood from patients with coronavirus disease 2019 (COVID-19) and healthy controls. *A*, Higher area under the curve (AUC) was identified for $CD8^+$ T cells, followed by $CD4^+$ T cells. *B*, Combined AUC of $CD8^+$ and $CD4^+$ T cells was 0.9029. Receiver operating characteristic (ROC) analysis of $CD4^+$ and $CD8^+$ T cells in peripheral blood from patients with severe vs mild-to-moderate illness. *C*, Higher AUC was identified for $CD4^+$ T cells, followed by $CD8^+$ T cells. *D*, Combined AUC of $CD8^+$ and $CD4^+$ T cells was 0.8810.

that the counts of T lymphocytes are related to the severity of SARS patients [4]. In addition, we found that severe patients admitted to ICU showed significant decreases in a count of lymphocyte subsets compared with mild-to-moderate patients, especially $CD3^+$, $CD4^+$, and $CD8^+$ T cells. This indicated that T lymphocytes were more suppressed in severe patients, which is consistent with recent studies [2, 3]. Researchers reported on 2 causes of T lymphopenia in SARS: sequestration into the lung of β-chemokine–recruited lymphocytes and interferon-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 vs 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 patients with mild-to-moderate vs severe illness. We speculated that Treg cells might not have a critical role in SARS-CoV-2 infection, and multicenter studies are needed to confirm our hypothesis.

According to the diagnosis and treatment protocol for pneumonia caused by a new coronavirus infection, published by the National Health Commission of China (trial version 7) [11], 2 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 $CD3^+$, $CD4^+$, 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-CoV-2 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 to the mild patient group in the third week; all of the patients with severe illness survived the disease. These results implied that the restoration of T lymphocytes was a favorable outcome.

Early diagnosis of COVID-19 and the identification of severe patients may facilitate the provision of appropriate supportive care. Based on our results, we selected $CD4^+$ and $CD8^+$ T cells as candidate diagnostic markers in the diagnosis of COVID-19 patients and the prediction of severe cases. Comparison between COVID-19 patients and HCs revealed that $CD4^+$ T cells had an AUC value of 0.7573, $CD8^+$ T cells had an AUC value of 0.8876, and the combination of $CD4^+$ and $CD8^+$ T cells had an AUC value of 0.9029, thus indicating that $CD4^+$ and $CD8^+$ T cells in peripheral blood have potential diagnostic value for COVID-19. The risk values of $CD8^+$ and $CD4^+$ T cells used for distinguishing between COVID-19 patients and HCs were 285.5/μL and 386.0/μL, respectively. Comparison between severe patients and mild-to-moderate patients revealed that $CD4^+$ T cells had an AUC value of 0.8666, $CD8^+$ T cells had an AUC value of 0.8618, and the combination of $CD4^+$ and $CD8^+$ T cells had an AUC value of 0.8810, thus demonstrating that $CD4^+$ and $CD8^+$ T cells also distinguished patients with severe illness from those with mild-to-moderate illness. The risk value of $CD8^+$ and $CD4^+$ T cells used for distinguishing between severe and mild-to-moderate cases was 103.5/μL and 238.5/μL, respectively. Recent studies have reported other prognostic factors such as neutrophil-to-lymphocyte ratio and neutrophil-to-$CD8^+$ T-cell ratio, which had a comparable performance with neutrophil-to-lymphocyte ratio in the ROC curve analysis [3, 12]. These researchers' kinetic analysis revealed that $CD8^+$ T cells were the major lymphocyte subset, which decreases in cell numbers during COVID-19 [3], consistent with our findings.

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 $CD8^+$ 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 cell percentage between mild-to-moderate and severe cases. This suggests that Treg cells may not have a critical role in SARS-CoV-2 infection. Of note, the sample size in our study was relatively small, and multicenter studies are needed to confirm our hypothesis. To conclude, we found the risk value of $CD8^+$ and $CD4^+$ T cells for the diagnosis of COVID-19 cases and the prediction of severe cases. Immunological characteristics of the lymphocyte subsets are important for exploring the mechanisms underlying SARS-CoV-2 infection. Large-scale multicenter clinical studies are needed to elucidate lymphocyte subsets in the immunological mechanisms of COVID-19.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. M. J. and Y. G. performed the experiments and drafted the manuscript. Q. L., Z. K. H., R. Z., and S. Y. L. discussed the advice from reviewers and revised the manuscript. A. P. L., J. M. L., and L. G. W. analyzed and interpreted the data and approved the final manuscript. All of the authors have read and approved the manuscript.

Acknowledgments. This study was supported by the Applied Research Training Program of Jiangxi Province (number 20181BBG78057); the Science and Technology Plan Project of Jiangxi Provincial Health Planning Commission (number 20165168); and the Science and Technology Research Project of the Jiangxi Provincial Department of Education (number GJJ150127).

Potential conflicts of interest. All authors: No reported conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Huang CL, Wang YM, Li XW, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet **2020**; 395:497–506.
2. Wan SX, Yi QJ, Fan SB, et al. Characteristics of lymphocyte subsets and cytokines in peripheral blood of 123 hospitalized patients with 2019 novel coronavirus pneumonia (NCP). medRxiv [Preprint]. 12 February **2020**. https://doi.org/10.1101/2020.02.10.20021832. Accessed 15 February 2020.
3. Liu J, Li SM, Liu J, et al. Longitudinal characteristics of lymphocyte responses and cytokine profiles in the peripheral blood of SARS-CoV-2 infected patients [manuscript published online ahead of print 18 April 2020]. EBioMedicine **2020**; 55:102763.
4. Li TS, Qiu ZF, Zhang LQ, et al. Significant changes of peripheral T lymphocyte subsets in patients with severe acute respiratory syndrome. J Infect Dis **2003**; 189:648–51.

5. Cui W, Fan Y, Wu W, Zhang F, Wang JY, Ni AP. Expression of lymphocytes and lymphocyte subsets in patients with severe acute respiratory syndrome. Clin Infect Dis **2003**; 37:857–9.
6. Huang KJ, Su IJ, Theron M, et al. An interferon-gamma-related cytokine storm in SARS patients. J Med Virol **2005**; 75:185–94.
7. Cecere TE, Todd SM, Leroith T. Regulatory T cells in arterivirus and coronavirus infections: do they protect against disease or enhance it? Viruses **2012**; 4:833–46.
8. Boettler T, Spangenberg HC, Neumann-Haefelin C, et al. T cells with a $CD4^+CD25^+$ regulatory phenotype suppress in vitro proliferation of virus-specific $CD8^+$ T cells during chronic hepatitis C virus infection. J Virol **2005**; 79:7860–7.
9. Marshall NA, Vickers MA, Barker RN. Regulatory T cells secreting IL-10 dominate the immune response to EBV latent membrane protein 1. J Immunol **2003**; 170:6183–9.
10. Yang G, Liu A, Xie Q, et al. Association of $CD4^+CD25^+Foxp3^+$ regulatory T cells with chronic activity and viral clearance in patients with hepatitis B. Int Immunol **2007**; 19:133–40.
11. National Health Commission of China. Diagnosis and treatment protocol for novel coronavirus pneumonia (trial version 7). China: National Health Commission, **2020**.
12. Liu JY, Liu Y, Xiang P, et al. Neutrophil-to-lymphocyte ratio predicts severe illnesspatients with 2019 novel coronavirus in the early stage. medRxiv [Preprint]. 12 February **2020**. https://doi.org/10.1101/2020.02.10.20021584. Accessed 18 February 2020.