

Clinical Observation of the Number of Lymphocytes and T Cell Subsets in Patients with COVID-19 at Different Stages

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Research Article

Keywords: COVID-19, dynamic changes, peripheral blood lymphocytes, T cell subsets, CD3+, CD4+, CD8+

Posted Date: May 19th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-30379/v1>

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Abstract

To explore the changes of lymphocytes and T cell subsets at different stages in patients with COVID-19. 86 patients with COVID-19 were enrolled, and the dynamic changes of peripheral blood lymphocytes and T cell subsets of CD3+, CD4+, and CD8+ were measured on admission, after treatment for 1 week, 2 weeks, and before discharge. There were no significant differences in the number of white blood cells and lymphocytes between admission and 2 weeks after treatment or before discharge in severe patients. The counts of CD3+, CD4+, and CD8+ T cells decreased significantly on admission. After 2 weeks of treatment, the CD3+ counts were significantly higher than that on admission. The CD4+ and CD8+ counts increased significantly after 1 week of treatment, and went up remarkably before discharge compared with that on admission. There was no significant difference in the number of CD3+ cells between the mild group and the control group on admission, but it was significantly lower in the severe group than that in the control group and the mild group. The CD4+ and CD8+ counts decreased significantly in both mild and severe patients on admission, and increased significantly before discharge. At the time of discharge, the CD4+ counts in the severe and mild groups were still significantly lower than in the control group, but there was no significant difference in CD8+ counts among the three groups. The counts of CD3+, CD4+, and CD8+ T cells in the patients with COVID-19 is significantly correlated with the short-term prognosis, and is more sensitive than lymphocytes. In the earliest stage, the numbers of CD4+ and CD8+ cells are more sensitive to early reduction and faster to late recovery.

Introduction

At present, more than three million people worldwide are infected with the Corona Virus Disease 2019 (COVID-19), and the number of deaths in some countries has increased ^{1,2,3}. The epidemic of COVID-19 in China has entered the middle and late stages, and now we mainly treat the hospitalized severe ill cases and screen the imported cases. We have accumulated some experience during the treatment process. It is important to identify those individuals with COVID-19 who will become critically ill and to treat them with the right methods. We observed that in severe COVID-19, although patients had lymphocytopenia, the lymphocytes were activated. However, the dynamic changes in the number and function of lymphocyte subsets, and what to do in the early stage of the severe illness have not yet been known. In the present study, some patients with severe and mild COVID-19 were selected to observe the dynamic changes of lymphocytes and T cell subsets during the treatment, and to observe the prognosis of the patients, in order to find new biomarkers to guide the therapy at the earliest stages of hospitalization^{4,5}.

Materials And Methods

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. All patients signed informed consent. The study was approved by the Ethics Committee of Renmin Hospital of Wuhan University (WDRY2020-K031).

Study design and participants

This is a two-center prospective cohort study, from February 1 to March 1, 2020. We included consecutive patients with laboratory confirmed COVID-19 who were admitted to Renmin Hospital of Wuhan University, Shouyi district and Optics Valley district, Wuhan city, Hubei province.

Procedures

5ml of venous blood was taken from the enrolled patients on admission, 1 week, 2 weeks and discharge. White blood cells (WBC), lymphocytes, and T cell subsets of CD3+, CD4+, and CD8+ were measured to observe the dynamic changes in different periods. Samples for T lymphocyte subsets were analyzed using FACSCanto-II cytometers (Becton Dickinson, San Jose, CA). The following human T lymphocyte subsets were determined (expressed as cells/ μ L): CD3+, CD4+, and CD8+ cells. Lymphocyte counts were performed as controls on 8 healthy individuals.

Critically ill patients were defined as those admitted to the intensive care unit (ICU) who required mechanical ventilation or had a fraction of inspired oxygen (FiO₂) of at least 60% or more^{6,7,8}. Laboratory confirmation of SARS-CoV-2 infection was performed by the clinical lab of our hospital authorized by Wuhan Center for disease control and prevention. The criteria for discharge were absence of fever for at least 3 days, substantial improvement in both lungs in chest CT, clinical remission of respiratory symptoms, and two throat-swab samples negative for SARS-CoV-2 RNA obtained at least 24 h apart⁹. All analyses were performed at a single laboratory. General clinical data of all patients were recorded, including a patient's history and physical examination and results of haematological, biochemical, radiological, and microbiological investigations, treatment (oxygen therapy, vasoconstrictive agents, antiviral agents, antibacterial agents, corticosteroids, and immunoglobulin). We got the counts of T lymphocyte subsets of the patients at different stages during hospitalization.

Data analysis

Statistical analysis was performed using IBM SPSS Version 20.0 software (IBM Corp., Armonk, NY, USA) and GraphPad Prism 5. The data were reported as N (%) or as median (interquartile range [IQR]) for categorical and continuous variables respectively. *P* values were calculated by chi-square test, Fisher's exact test, or Mann-Whitney U test, Kruskal-Wallis test where appropriate. A value of *P* < 0.05 was considered statistically significant.

Results

A total of 86 new COVID-19 patients were enrolled, including 44 severe patients, 42 mild patients, and 8 healthy volunteers as controls. Mild patients were treated in the common isolation ward, while severe patients were treated in the ICU isolation ward. Among the 44 patients with severe diseases, 4 died rapidly after admission to ICU, 4 patients had incomplete data, and 4 patients requested to withdraw from the study voluntarily. A total of 32 patients with severe diseases completed the research in the end. Two critically ill patients died between the first week and the second week, and two died between the second week and discharge. Among the mild patients, 3 patients had incomplete data, 5 patients withdrew voluntarily, 3 patients became severe and were transferred to ICU, and a total of 31 patients completed the study. The data of enrolled patients are shown in table 1 and table 2.

Table 1 Demographics and baseline characteristics, comorbidities of enrolled patients

	Severe patients (n=32)	Mild patients (n=31)	Control group (n=8)	<i>P</i> -values
Demographics and clinical characteristics				
Age, years	52.2[45.8-76.3]	49.6[38.5-74.3]	49.3[43.6-65.2]	<0.01
Sex				
Female	12(38%)	13(42%)	3(38%)	..
Male	20(63%)	18(58%)	5(63%)	..
Exposure				
Exposure to Huanan sea food market	4(13%)	1(3%)	0(0%)	0.173
Exposure to patients	8(25%)	10(32%)	0(0%)	0.524
Comorbidities				
Hypertension	8(25%)	9(29%)	1(13%)	0.718
Coronary heart disease	4(13%)	3(10%)	2(25%)	0.722
Chronic pulmonary disease	6(19%)	4(13%)	1(13%)	0.525

Cerebrovascular disease	6(19%)	6(19%)	1(13%)	0.951
Diabetes	5(16%)	6(19%)	1(13%)	0.697
Malignancy	2(6%)	1(3%)	0(0%)	0.573
Chronic kidney disease	2(6%)	2(6%)	0(0%)	0.974
Current Smoker	8(25%)	10(32%)	3(38%)	0.524
none	9(28)	12(39%)	4(50%)	0.373

P value: severe patients vs. mild patients.

Table 2 Symptoms, blood Test, chest x-ray(or CT) on admission and treatments of patients

	Severe patients (n=32)	Mild patients (n=31)	<i>P</i> -values
Symptoms			
fever	30(94%)	26(81%)	0.212
Cough	28(88%)	20(63%)	0.032
Dyspnoea	28(88%)	16(52%)	0.002
Chills	28(88%)	14(44%)	<0.0001
Runny nose	26(81%)	14(44%)	0.003
Blocked nose	28(88%)	16(52%)	0.002
Sore throat	25(78%)	12(39%)	0.001
Chest discomfort	16(50%)	8(26%)	0.048
Diarrhea	6(19%)	3(10%)	0.304
Myalgia	16(50%)	12(39%)	0.367
Malaise	10(31%)	8(26%)	0.633
Duration of symptoms before admission	4(0-8)	6(4-10)	0.357
Blood tests on admission			
White blood cell count, × 10 ⁹ / L	6.9(4.2-10.3)	4.8(3.8-9.4)	<0.0001
Lymphocyte count, × 10 ⁹ / L	1.2(0.8-1.9)	1.6(0.9-2.1)	<0.001
Haemoglobin, g/L	124(110-129)	126(112-135)	0.35
Platelet count, × 10 ⁹ / L	230(130-280)	280(140-289)	<0.001
IL-6, pg/mL	7.4(4.8-9.2)	3.6(2.9-5.9)	<0.0001
Chest x-ray and CT findings			
Unilateral pneumonia	0(0%)	6(19%)	0.009
Bilateral pneumonia	32(100%)	16(52%)	<0.0001
Multiple mottling and ground-glass opacity	32(100%)	14(44%)	<0.0001
Treatment			
Oxygen therapy	32(100%)	16(19)	<0.0001
Non-invasive (ie, face mask)	12(38%)	16(19)	0.260
Invasive	20(63%)	0(0%)	<0.0001
CRRT	6(19%)	0(0%)	0.011
Antibiotic treatment	32(100%)	16(19%)	<0.0001
Antifungal treatment	2(6%)	0(0%)	0.157
Antiviral treatment	32(100%)	31(100%)	..
Other treatments			
Glucocorticoids	6(19%) 0(0%)	0(0%)	0.011
Intravenous immunoglobulin therapy	4(13%) 0(0%)	0(0%)	0.042

Data is presented as numbers (%) or median (IQR). *P* values were calculated by chi-square test, Fisher's exact test, or Mann-Whitney U test, where appropriate.

CRRT= continuous renal replacement therapy.

There were no significant differences in the number of WBC and lymphocytes on admission of severe patients compared with that after 2 weeks of treatment and before discharge. No significant differences were found in the CD3+ counts between 1 week and admission, but there was a significant increase ($P<0.01$) in the counts between 2 weeks and admission, and it continued to increase before discharge. CD4+ and CD8+ counts were increased significantly at 1 week compared with that on admission ($P<0.05$). See Figure 1.

Figure 2 showed that there was no difference in WBC counts among the three groups on admission, and WBC counts decreased significantly in severe patients before discharge ($P<0.05$), while there was no difference in the rest. However, there was a significant decrease in the number of lymphocytes in the severe group compared with other groups (all $P<0.01$). Before discharge, there was no difference in lymphocytes counts among the three groups. There was no significant difference in the CD3+ counts between the mild group and the control group on admission, but the CD3+ counts in the severe group were significantly lower than that in the control group and the mild group ($P<0.001$). There was no significant difference in the CD3+ counts between the mild group and the control group before discharge, but the CD3+ counts in the severe group were significantly lower than that in the other two groups ($P<0.001$). On admission, the CD4+ counts in the mild and severe groups decreased significantly compared with the control group, and gradually increased after treatment. There was no significant difference in the CD4+ counts between the severe group and the mild group before discharge, but it still decreased significantly compared with the control group ($P<0.001$). The CD8+ counts decreased significantly in both mild and severe patients on admission, but there was no significant difference between the severe and the mild group. Before discharge, the CD8+ counts recovered well and there were no significant differences among the three groups.

Discussion

The critically ill COVID-19 have some special characteristics in common: autopsy results show that the spleen and multiple lymph nodes atrophy accompanied by a significant decrease in the number of lymphocytes, the injured lung tissue has a large number of monocytes and macrophages infiltration, but the number of lymphocytes is less. In terms of clinical symptoms, severe patients usually experience sudden deterioration 7–14 days after infection, which may be related to the cytokine storm. The result of peripheral venous blood test showed that lymphocytes were mostly decreased. With the cytokine storm, the patients showed unstable symptoms, accompanied by bacterial infection and coagulation disorders, and some patients had poor clinical outcomes *10,11,12,13,14*.

Now it seems that there are widespread infections in many countries, and the virus is likely to coexist with us for a long time. The key to the treatment of COVID-19 is to identify the patients who are likely to convert to severe infection at an earliest stage, and to give effective treatment to prevent the patients from progressing to acute respiratory distress syndrome (ARDS). At present, there is no simple and feasible biomarker or imaging test that can identify high-risk patients in early stage, thus saving ICU resources and improving the prognosis of patients. The conventional WBC and lymphocyte counts are affected by multiple factors, so they are not accurate and sensitive enough. Lung CT examination can see the inflammatory infiltration of the lung tissues of the patients, but the symptoms of the patients are more serious when there is obvious infiltration shadow in the double lungs. It has been widely observed that the decline of lymphocytes is significantly correlated with the poor prognosis of patients, but the dynamic changes of lymphocytes and their subsets at different stages and the changes of lymphocyte function in different subsets have not been observed *15,16,17,18,19*.

Our study dynamically observed the changes of peripheral blood lymphocytes and T cell subsets in mild and severe patients. We found that the CD4+ counts of mild and severe patients decreased significantly compared with the control group ($P<0.001$) on admission. Before discharge, the CD4+ counts in these patients increased significantly compared with that on admission. However, the CD4+ counts in the severe group were still significantly decrease compared with the control group. The CD8+ counts also decreased significantly in mild patients ($P<0.01$) on admission, and the decline was more pronounced in severe patients, while they all recovered to normal before discharge. After infection, T lymphocyte subsets immediately

reduced significantly, which are more sensitive than WBC and lymphocytes, and are unaffected by other factors. The lower lymphocyte subsets are directly related to the poor prognosis of patients with COVID-1920.

The total number of leukocytes and lymphocytes were affected by many factors. In the early stage, some severe patients were infected with secondary leukocytes elevation. There was no significant difference in leukocytes counts between the mild group and the control group on admission, which could not be used as an earliest screening index. Therefore, the measurement of the number of lymphocytes and their subsets may be a more effective and sensitive indicator for early identification. While we are concerned about the number of lymphocytes and their subsets, we should also pay attention to the changes of the function of lymphocyte subsets. In some cases, the counts of peripheral CD4+ and CD8+ T cells were substantially reduced, while their status was hyperactivated. The excessive hyperfunction and lower number of the lymphocytes may be the source of cytokine storm. Our results imply that overactivation of lymphocyte subsets accounts for the severe immune injury in these patients.

Drugs targeting lymphocyte proliferation or apoptosis (IL-7, PD1/PD-L1 inhibitors) could help to prevent lymphopenia or improve lymphocyte counts in severe patients. It is the key point to give the immunoregulatory treatment in the earliest stage of the patients with COVID-19. We need to improve the counts of lymphocytes and the subsets to prevent cytokine storm. Infusion of immunoglobulin, low dose hormone therapy, and traditional Chinese medicine conditioning are the choices of the clinical treatment 27. On February 14, our guidelines explicitly recommended the use of immunoglobulin therapy in critically ill patients. We recommend traditional Chinese medicine to the mild patients, such as lianhua qingwen capsule, and pneumonia prescription 1. These methods have achieved good outcomes. One of the reasons why traditional Chinese medicine is effective is related to the improvement of patients' immune function. Anticytokine therapy alone may be unpromising, anti-IL-6 is not effective, and blood replacement therapy (artificial liver therapy) is not effective, because immune activation may be involved in multiple-factors22.

This study suggests that the detection of the number of peripheral blood lymphocytes and subsets in patients with COVID-19 is a sensitive indicator to understand the progress and direction of the disease. We should pay attention to increase the number of lymphocytes and subsets and improve the cellular function in earliest stage.

Data availability

Data produced and processed in this study are included in the published article and the supplementary files. The datasets can be acquired from the corresponding author upon appropriate purposes.

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Declarations

Acknowledgments

We thank all patients and their families involved in the study.

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Contributions

J. B and H. Z collected the epidemiological and clinical data. J.B and B.S summarised all data and drafted the manuscript. J.B and H. Z revised the final manuscript.

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Ethics declarations

Competing interests

We declare no competing interests.

Additional information

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Figures

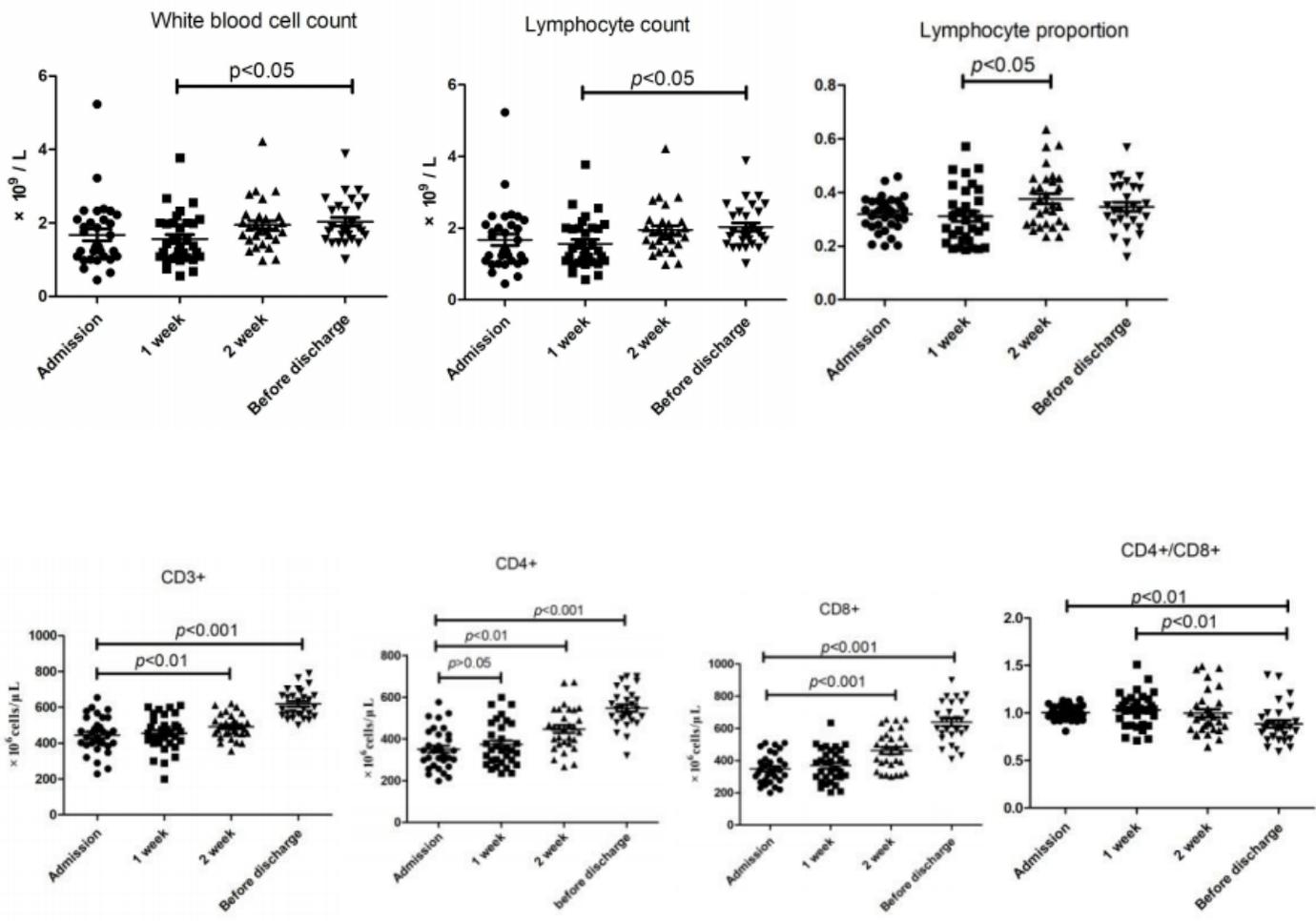


Figure 1

Counts of White blood cells, Lymphocytes and their subsets in critically ill patients with COVID-19 at different stages.

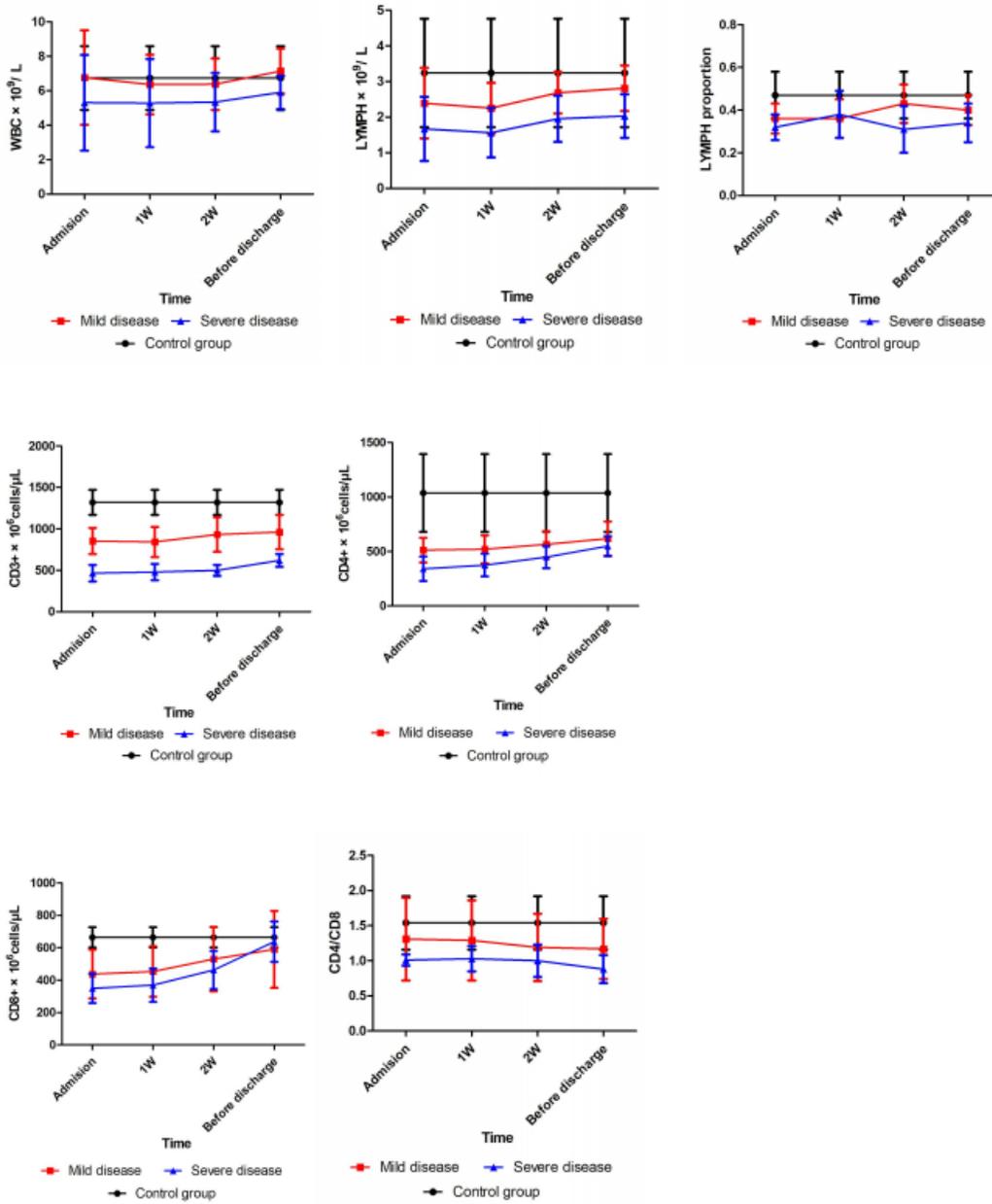


Figure 2

Counts of White blood cells, Lymphocytes and their subsets in three groups at different stages. Comparison of WBC, lymphocytes and subsets among severe patients with COVID-19 (n=32), mild patients with COVID-19(n=31), and healthy controls(n=8). WBC=white blood cells; LYMPH= lymphocytes