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Analysis of the expression and significance of lymphocyte ratio in different stages and clinical types of COVID-19

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ABSTRACT

This study aimed to analyze the expression of lymphocyte ratio (LY%) in different stages and clinical staging of COVID-19 and explore the relationship between peripheral blood lymphocyte (PBL) ratio and COVID-19 severity to provide reference for early intervention. For this purpose, a total of 125 patients with COVID-19 admitted to Hebei Provincial People's Hospital from February 1, 2020, to March 1, 2022, were reviewed and divided into moderate, severe, and critical groups by the severity to analyze and compare peripheral lymphocyte ratios of patients with different clinical typing. Results showed that lymphocyte count, lymphocyte percentage, CD3+ T-lymphocyte count, CD4+ T-lymphocyte count, and CD8+ T-lymphocyte count all decreased gradually with increasing severity ($F = 27.84$, $P<0.05$; $F = 15.28$, $P<0.05$; $F = 46.12$, $P<0.05$; $F = 34.65$, $P<0.05$); the absolute numbers of CD3+, CD4+ and CD8+ cells in peripheral blood were higher in the recovery phase than in the acute phase ($P<0.05$). In conclusion, COVID-19 may cause a decrease in the number of lymphocytes, and the decrease in the number of lymphocytes and T-lymphocyte subsets may predict the severity of the disease. The fewer lymphocytes there are, the more likely they are to progress to the severe type and the worse the prognosis.

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Introduction

Coronavirus disease 2019, referred to as "COVID-19", is a severe acute respiratory tract infectious disease caused by β genus COVID-19 (1). It is highly infectious through respiratory tract droplets and close contact (2). At the initial stage, COVID-19 is characterized by fever, dry cough, malaise, and then respiratory distress, which can progress to acute respiratory distress syndrome or septic shock, or even death in severe cases (3). In the face of such huge difficulty of treatment and high risk of death, early diagnosis, effective identification, and interruption of the progress from mild to severe conditions are the keys to reducing morbidity and mortality.

COVID-19 invades and damages host cells after binding to human angiotensin-converting enzyme 2 (ACE2) mediated by its surface spike protein (4), causing inflammatory lesions in the lung and also damage to the heart, digestive system and nervous system (5). In addition, it was found that COVID-19 attacks the human immune system, and consequently, shortened lymphocyte half-life, increased apoptosis, and increased secretion of adrenocorticotropic hormone leads to a decrease in the PBL base, with a progressive decrease in PBL seen in severe cases (6-8). Lymphocytes are an important component of the immune system, and it is of great clinical significance to explore the expression of the PBL ratio in different stages and clinical staging as well as its relationship with the severity of COVID-19. To this end, this study was conducted to dynamically analyze lymphocytes and their subpopulations in COVID-19 patients, in anticipation of providing a new reference for the diagnosis and evaluation of COVID-19.

Materials and Methods

General data

A total of 125 patients with COVID-19 admitted to Hebei Provincial People's Hospital from February 1, 2020, to March 1, 2022, were pooled and divided into moderate, severe, and critical groups according to the *Diagnosis and Treatment Protocol for COVID-19 (Trial Version 5)* (9) to compare their basic information, previous underlying conditions, and laboratory tests.

Clinical typing of COVID-19

All diagnosed patients were clinically staged according to the *Diagnosis and Treatment Protocol for COVID-19*. (I) Moderate: fever, respiratory tract symptoms, and pneumonia on imaging. (II) Severe: having any of the following symptoms: shortness of breath, respiratory rate ≥ 30 bpm; oxygen saturation ≤ 93% at rest; PaO2/PiO2 ≤ 300 mmHg (1 mmHg = 0.133 kPa). (III) Critical: having one of the following conditions: respiratory failure with the need for mechanical ventilation; shock; other organ failure requiring ICU treatment.

Specimen collection and laboratory testing

Vacuum tubes containing anticoagulant and procoagulant were respectively used to collect fasting venous blood from patients early in the morning. Of them, EDTA-K2 anticoagulated venous blood specimens were collected to detect routine blood parameters and the absolute number of T-cell subsets. Then the routine blood parameters, including white blood cell count, lymphocyte count, neutrophil count and platelet count, were tested by a blood cell ana-

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lyzer. The T cell subsets were detected by flow cytometry. Specifically, 50 μL of EDTA-K2 anticoagulated blood was placed in a flow cytometry tube, added with 10 μL of TriTEST CD3-PerCP/CD4-FITC/CD8-PE antibody, incubated for 15 min at room temperature and protected from light, then mixed with 2 mL of hemolysin, incubated for 15 min, centrifuged, washed twice with PBS, and detected by flow cytometry.

Statistical processing

Data were analyzed using SPSS 19.0. Normal measures of continuous variables were expressed as x±s, while categorical variables were statistically described by frequency (composition ratio). The measurement data were compared between groups using the independent samples t-test whereas the count data were tested with the X2 test. P<0.05 was considered a statistically significant difference.

Results

Basic information

A total of 125 confirmed COVID-19 cases were enrolled, 68 males and 57 females, aged (59.54±16.58) years. The age range was (53.35±14.52) years in the moderate group, (58.45±15.46) years in the severe group and (68.64±16.32) years in the critical group, with an increasing trend; the t-test was performed for pairwise comparison (P<0.05), which was statistically significant. Besides, the highest body temperature range was (38.65±0.75) ℃ in the moderate group, (38.68±0.76) ℃ in the severe group, and (39.14±0.88) ℃ in the critical group, indicating a sequential increase. However, there was no statistically significant difference in body temperature between the moderate and the severe groups, P>0.05. In contrast, the differences between the moderate and the critical groups and between the severe and the critical groups were statistically significant, P<0.05, as shown in Table 1.

Comparison of peripheral blood laboratory tests in patients with different clinical staging

In the three groups, there was a statistical difference in neutrophils, lymphocytes, and lymphocytes between the moderate and the severe groups (P<0.05), a statistical difference in leukocytes, neutrophils, and lymphocytes between the moderate and the critical groups (P<0.05), and a statistical difference in platelets and lymphocytes between the severe and the critical groups (P<0.05) (Table 2).

Levels of lymphocytes and various subpopulations in patients with different clinical staging

Lymphocyte counts and lymphocyte ratios decreased with disease progression in all three groups, with statistically significant differences overall (P<0.05) and between each of the two groups (P<0.05). The counts of all subgroups gradually decreased with disease progression (P<0.05), and CD3+ count, CD4+ count, and CD8+ count significantly reduced between every two groups (P<0.05), with the highest in the moderate group and the lowest in the critical group (Figure 1).

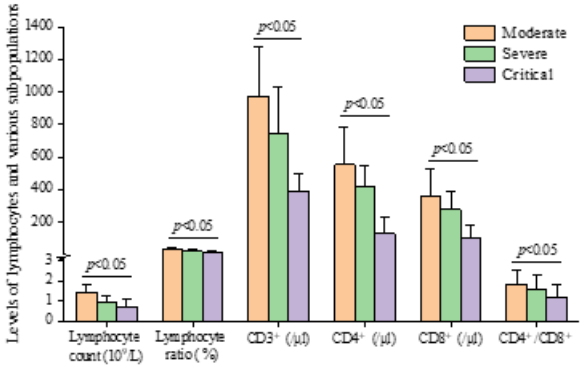

Figure 1. Comparison of peripheral blood laboratory test indexes in patients with different clinical.

Table 1. Basic information on 125 patients with COVID-19.

Clinical features	Total cases (125)	Moderate (61)	Severe (45)	Critical (19)
Age	59.54± 16.58	53.35±14.52	58.45±15.46	68.64±16.32
Sex				
Male	68 (54.40)	33 (54.09)	23 (51.11)	12 (63.15)
Female	57 (45.60)	28 (45.91)	22 (48.89)	7 (36.85)
Length of stay	9.05±5.42	8.82±5.21	8.86±5.35	9.58±5.94
Days with fever	10.06±6.09	9.34±5.46	11.72±6.82	11.78±6.91
T_{max}	38.53±0.72	38.65±0.75	38.68±0.76	39.14±0.88
Past medical history				
Underlying disease	61 (48.80)	23 (37.71)	25 (55.56)	13 (68.42)
No underlying disease	64 (51.20)	38 (62.29)	20 (44.44)	6 (31.58)

Table 2. Comparison of peripheral blood laboratory test indexes in patients with different clinical staging.

Indicators	Normal range	Total	Moderate	Severe	Critical	P1	P2	P3
Leukocytes (10^9/L)	3.5-9.5	5.33±3.19	4.75±1.82	5.38±3.51	6.56±4.07	0.29	<0.05	0.18
Blood platelets (10^9/L)	125-350	182.54±60.34	185.48±62.28	206.59±68.48	160.25±56.10	0.07	0.25	<0.05
Neutrophils (10^9/L)	1.8-6.3	4.83±11.05	3.12±1.64	4.25±3.02	9.56±3.10	<0.05	<0.05	0.14
Lymphocytes (10^9/L)	1.1-3.2	0.95±0.31	1.46±0.35	0.98±0.32	0.70±0.42	<0.05	<0.05	<0.05
Mononuclear cells (10^9/L)	0.1-0.6	0.37±0.22	0.39±0.25	0.27±0.16	0.35±0.20	0.37	0.75	0.35

Note: P1: moderate VS severe; P2: moderate VS critical; P3: severe VS critical.

Analysis of the changes in RR, SpO2, PaO2/FiO2 and their correlation with lymphocytes

Compared with general symptomatic patients and critically ill patients, severe patients had higher RR and lower SpO2, PaO2 /FiO2 (P<0.05) (Fig2). The correlation analysis showed that SpO2 and PaO2 /FiO2 were positively correlated with lymphocyte count (r = 0. 429, 0.296, P<0.05).

Comparison of T-cell subsets in COVID-19 patients in the acute and recovery phases

In the acute phase, CD3+ cells were reduced in 63 COVID-19 patients (50.40%), CD4+ cells were reduced in 67 patients (53.60%), and CD8+ cells were reduced in 52 patients (41.60%). The comparison between acute and recovery T cell subsets showed that the absolute numbers of CD3+, CD4+ and CD8+ cells in the peripheral blood of COVID-19 patients in the recovery period were higher than those in the acute period (P<0.05) Figure 3).

Discussion

Human coronaviruses are classified into α, β, γ and δ genera. COVID-19, a β-genus coronavirus, contains an envelope and round or oval particles, 60-140 nm in diameter. It is polymorphic and has more than 85% homology between its genome and bat SARS-like COVID-19, so that it can be transmitted between humans (10,11). The condition is clinically characterized by acute respiratory infections and is classified into moderate, severe, and critical types. Furthermore, severe and critical COVID-19 patients may develop respiratory failure within a short period. At present, the mortality rate of COVID-19 is about 3. 5% in China. Clinical observation revealed that some patients especially severe patients showed decreased lymphocyte count in blood routine (12-15). However, the variability of lymphocyte counts in patients of different periods and subtypes is still unknown, hence the significance of this study.

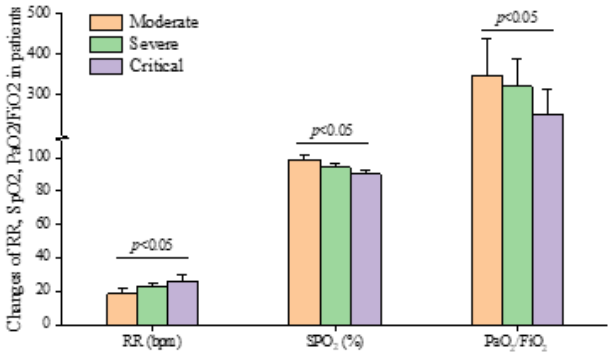

Figure 2. Changes of RR, SpO2, PaO2/FiO2 in patients with different clinical staging.

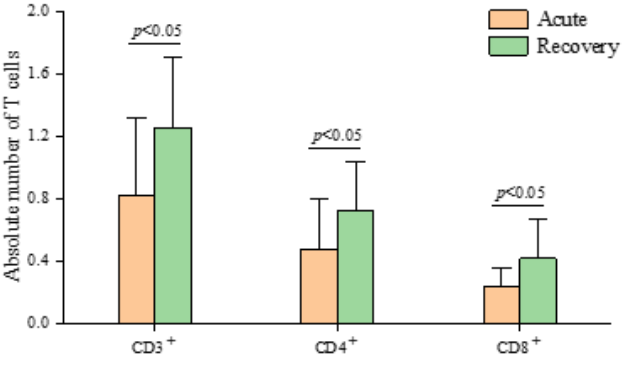

Figure 2. The absolute number of T cells in the acute phase compared with the recovery phase.

In the analysis for age, it was found that the older the patients with the moderate, severe and critical symptoms, the more severe their condition was. And pairwise comparison showed a statistical difference (P<0.05). This indicates that older patients are more likely to progress to severe pneumonia, possibly because they have more underlying diseases and degraded immune function. In addition, the mean values of body temperature in the three groups increased with the severity of the disease. Therefore, critically ill patients are more likely to have higher body temperature during the course of the disease, and their body temperature changes need to be closely observed.

Lymphocyte subpopulation analysis is a vital index for detecting cellular and humoral immune functions, hence reflecting the immune status and body balance. It is important for observing the efficacy and determining the clinical prognosis (16) as it can assist in diagnosing certain diseases and analyzing pathogenesis. In this study, the lymphocyte count, lymphocyte percentage, CD3+ T lymphocyte count, CD4+ T lymphocyte count, and CD8+ T lymphocyte count were confirmed to gradually decrease with increasing disease severity. Consistent with the previously reported findings (17,18), this suggests that COVID-19 may mainly attack lymphocytes in the body and cause lymphocytopenia. Upon entry into the body, COVID-19 may undergo antigen presentation to form immune complexes that cause lymphocytes in the circulating pool to accumulate toward tissues, resulting in a decrease in lymphocyte counts in peripheral blood (19). Therefore, the possibility of developing critical cases in those with persistent lymphocytopenia should be highly alerted and early intervention is recommended to reduce the morbidity and mortality of critically ill patients. Additionally, this study showed an increase in RR and a decrease in SpO2, PaO2 /FiO2 (P<0.05) in the critical group compared with the moderate and severe groups. Correlation analysis showed that SpO2 and PaO2 /FiO2 were positively correlated with the number of lymphocytes (P<0.05).

Changes in PBL can be used as a basis for early diagnosis of COVID-19 (20). In the present study, the absolute number of CD3+ cells decreased in 50.40% of acute COVID-19 patients, CD4+ cells in 53.60% of the patients, and CD8+ cells in 41.60% of the patients. By tracing to its source, viral infection causes activation of immune cells and their involvement in antiviral resistance, leading to cellular damage and apoptosis, and immune cell adhesion molecules move circulating pool cells into tissues, causing a decrease in PBL. Nevertheless, CD3+, CD4+, and CD8+ cells were significantly higher during the recovery period, indicating that the damage to T lymphocytes by COVID-19 is reversible (21).

In conclusion, the PBL count in COVID-19 patients can be an effective biological indicator for early prediction of disease staging to help early clinical intervention, reduce the probability of progression from moderate conditions to severe conditions, and achieve a better prognosis.

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