Dysregulation of immune response in patients with COVID-19 in Wuhan, China

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Main points of this manuscript: Dysregulation of immune response, especially T lymphocytes, might be highly involved in the pathological process of COVID-19. Surveillance of NLR and lymphocyte subsets is helpful in the early screening of critical illness, diagnosis and treatment of COVID-19.

Abstract

Background

In December 2019, coronavirus disease 2019 (COVID-19) emerged in Wuhan and rapidly spread throughout China.

Methods

Demographic and clinical data of all confirmed cases with COVID-19 on admission at Tongji Hospital from January 10 to February 12, 2020, were collected and analyzed. The data of laboratory examinations, including peripheral lymphocyte subsets, were analyzed and compared between severe and non-severe patients.

Results

Of the 452 patients with COVID-19 recruited, 286 were diagnosed as severe infection. The median age was 58 years and 235 were male. The most common symptoms were fever, shortness of breath, expectoration, fatigue, dry cough and myalgia. Severe cases tend to have lower lymphocytes counts, higher leukocytes counts and neutrophil-lymphocyte-ratio (NLR), as well as lower percentages of monocytes, eosinophils, and basophils. Most of severe cases demonstrated elevated levels of infection-related biomarkers and inflammatory cytokines. The number of T cells significantly decreased, and more hampered in severe cases. Both helper T cells and suppressor T cells in patients with COVID-19 were below normal levels, and lower level of helper T cells in severe group. The percentage of naïve helper T cells increased and memory helper T cells decreased in severe cases. Patients with COVID-19 also have lower level of regulatory T cells, and more obviously damaged in severe cases.

Conclusions

The novel coronavirus might mainly act on lymphocytes, especially T lymphocytes. Surveillance of NLR and lymphocyte subsets is helpful in the early screening of critical illness, diagnosis and treatment of COVID-19.

Keywords: Lymphocyte subsets; T lymphocyte; immune response; COVID-19

Introduction

The outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), that first emerged in Wuhan in December 2019, has rapidly spread throughout China in the past two months.[1, 2] Considering the ongoing outbreak in China and fast worldwide spread of coronavirus disease 2019 (COVID-19), infected by SARS-CoV-2, it has led to the declaration of Public Health Emergency of International Concern by the World Health Organization (WHO) on 30 January 2020.[3] As of Feb 16, 2020, a total of 58182 laboratory-confirmed cases, has been identified in China (primarily in Wuhan), with 1696 fatal cases, according to the data from Chinese government official reports.[2]

It has been reported that the COVID-19 was more likely to occur in older men with comorbidities[4-6], who have weaker immune functions. As a new type of highly contagious disease in human, the pathophysiology of unusually high pathogenicity for COVID-19 has not been completely understood yet. Several studies have shown that increased amounts of proinflammatory cytokines in serum were associated with pulmonary inflammation and extensive lung damage in SARS[7] and MERS-CoV infection[8], and recently in COVID-19[5]. However, little is known about lymphocyte subsets and the immune response of patients with COVID-19.

This retrospective, single-center study aimed to analyze the expression of infection related biomarkers, inflammatory cytokines and lymphocyte subsets by flow cytometry in laboratory-confirmed cases, and compare the difference between severe cases and non-severe ones.

Methods

Study design and participants

We retrospectively recruited totally 452 patients with COVID-19 from January 10 to February 12, 2020, at Tongji hospital, the largest comprehensive medical treatment center of the central China and "the specific hospital for the treatment of severe patients with COVID-19 in Wuhan" designated by the government. The study was performed in accordance with Tongji Hospital Ethics Committee (IRB ID: TJ-C20200121). Written informed consent was waived by the Ethics Commission of the designated hospital for emerging infectious disease.

The severity of COVID-19 was judged according to the Fifth Revised Trial Version of the Novel Coronavirus Pneumonia Diagnosis and Treatment Guidance.[9] Those who met the criterion as follows were defined as severe-type: 1. Respiratory distress with the respiratory rate over 30 per minute; 2. Oxygen saturation ≤ 93% in the resting state; 3. Arterial blood oxygen partial pressure (PaO2) / oxygen concentration (FiO2) ≤300mmHg.

Data Collection

Data including demographic data, medical history, symptoms, signs, and laboratory findings were collected from patients' medical record. Laboratory results included blood routine, lymphocyte subsets, infection-related biomarkers, inflammatory cytokines, immunoglobulins and complement proteins. The total number of lymphocytes in peripheral blood was counted by hemocytometer. Lymphocyte subsets percentage were analyzed with FACSCanto flow cytometer for those COVID-19 patients on admission.[10] The absolute numbers of different lymphocyte subsets were calculated by multiplying the percentages with total lymphocyte count. PMA/ionomycin-stimulated lymphocyte function assay was performed as described

previously.[11] The percentages of IFN-γ positive cells in different cell subsets were defined as the function of them. The data were reviewed by a trained team of physicians in Tongji Hospital.

Real-Time Reverse Transcription Polymerase Chain Reaction Assay

A confirmed COVID-19 case was defined as positive for real-time reverse-transcriptase polymerase-chain reaction (RT-PCR) assay for nasal and pharyngeal swab specimens according to the WHO guidance. On receipt of the samples, viral RNA extraction was performed using a Magnetic viral RNA/DNA extraction Kit on PAN9600 Automated Nucleic Acid Extraction System (Tianlong, Xi'an, China), according to the manufacturer's instructions, followed by polymerase-chain-reaction (PCR) screening for the presence of specific 2019-nCoV with a commercial kit (Tianlong, Xi'an, China) in a volume of 25 μL PCR mixture containing 17.5 μL reaction solution, 1.5 μL probes, 1.5 μL Taq, and 5 μL nucleic acid. Conditions for the amplifications include reverse transcription at 50°C for 30 min, pre-denaturation at 95°C for 10 min, followed by 5 cycles of 94°C for 15 s, 50°C for 30 s and 72°C for 30 s, and 40 cycles of 94°C for 10 s and 58°C for 30 s for fluoresce detection. A cycle threshold value (Ct-value) ≤ 37 was defined as a positive test, which was based on the recommendation by the National Institute for Viral Disease Control and Prevention (China).

Statistical Analysis

We described the categorical variables as frequency rates and percentages, and continuous variables as mean and standard deviation (SD), median and interquartile range (IQR) values. Independent group t tests were used for the comparison of means for continuous variables that were normally distributed; conversely, the Mann-Whitney U test was used for continuous variables not normally distributed. Proportions for categorical variables were compared using the $\chi 2$ test. All statistical analyses were performed using SPSS (Statistical Package for the Social Sciences) version 20.0 software (SPSS Inc.). Two-sided P-values of less than 0.05 were considered statistically significant.

Results

Demographic and clinical characteristics of patients infected with COVID-19

By February 12, 2020, 452 consecutive patients with COVID-19 on admission to hospitalization at Tongji Hospital were recruited in this study, 286 (63.3%) of whom were clinically diagnosed as severe infection. In total, the median age was 58 years (IQR, 47-67; range, 22-95 years), and 235 (52.0%) were men. Compared with non-severe patients, severe patients were significantly older (median age, 61 years [IQR, 51-69] vs 53 years [IQR, 41-62]; $P < 0.001$). The proportion of men in the severe group (54.2% men) had no significant difference with the non-severe group. Of the 452 patients with COVID-19, 201 (44.0%) patients had chronic diseases (i.e., hypertension, diabetes, chronic obstructive pulmonary disease), and a higher percentage in the severe cases (146[51.0%]) than the mild cases (55[33.1%]). And those severe patients were significantly more likely to have concomitant hypertension and cardiovascular diseases (36.7% vs 18.1%; $P < 0.001$; 8.4% vs 1.8%; P =

0.004; respectively). The most common symptoms were fever (92.6%), shortness of breath (50.8%), expectoration (41.4%), fatigue (46.4%), dry cough (33.3%) and myalgia (21.4%). Moreover, severe patients were significantly more likely to have short of breath and fatigue (58.4% vs 39.2%; P < 0.001; 51.4% vs 39.2%; P = 0.014; respectively) than non-severe patients.

Blood cell counts, infection related biomarkers, inflammatory cytokines, immunoglobulins and complement proteins in patients with COVID-19

Table 2 presents the laboratory findings in patients with COVID-19. Among 452 patients who underwent laboratory examinations on admission, most of them tended to have lymphopenia, higher infection-related biomarkers (i.e. procalcitonin, erythrocyte sedimentation rate, serum ferritin, and C-reactive protein) and several elevated inflammatory cytokines (i.e. tumor necrosis factor (TNF)- α , interleukin (IL)-2R and IL-6), and there were numerous differences in blood cell counts and infection related biomarkers between severe group and non-severe group. Severe cases had higher leukocyte (5.6 vs 4.9 $\times 10^9$; P < 0.001) and neutrophil (4.3 vs 3.2 $\times 10^9$; P < 0.001) counts, lower lymphocytes counts (0.8 vs 1.0 $\times 10^9$; P < 0.001), higher neutrophil-to-lymphocyte ratio (NLR) (5.5 vs 3.2; P < 0.001) as well as lower percentages of monocytes (6.6 vs 8.4 %; P < 0.001), eosinophils (0.0 vs 0.2 %; P < 0.001), and basophils (0.1 vs 0.2 %; P = 0.015). Compared with non-severe group, most of severe cases demonstrated elevated levels of infection-related biomarkers, including procalcitonin (0.1 vs 0.05 ng/mL; P < 0.001), serum ferritin (800.4 vs 523.7 ng/mL; P < 0.001), and C-reactive protein (57.9 vs 33.2 mg/L; P < 0.001). A bunch of inflammatory cytokines were also elevated in severe cases than the non-severe ones, including interleukin (IL)-2R (757.0 vs 663.5 U/mL; P = 0.001), IL-6 (25.2 vs 13.3 pg/mL; P < 0.001), IL-8 (18.4

vs 13.7 pg/mL; P < 0.001), IL-10 (6.6 vs 5.0 pg/mL; P < 0.001), and TNF-α (8.7 vs 8.4 pg/mL; P = 0.037). Immunoglobulins (IgA, IgG and IgM) and complement proteins (C3 and C4) in patients with COVID-19 were within normal range. There were no significant differences in the levels of IgA, IgG, and complement proteins C3 or C4 between the mild and severe groups, while IgM slightly decreased in severe ones.

Lymphocyte subset analysis in patients with COVID-19

Lymphocyte subsets were analyzed in 44 patients with COVID-19 on admission (Table 3). The total number of B cells, T cells and NK cells significantly decreased in patients with COVID-19 (852.9 /uL), and more evident in the severe cases (743.6 vs 1020.1 /uL; P = 0.032) compared to the non-severe group. The mean values of the three main subsets of lymphocytes were generally decreased in patients with COVID-19, as T cells and NK cells below normal levels, and B cells within the lower level of normal range. T cells were shown to be more affected by SARS-CoV-2 as T cell count was nearly half the lower reference limit, and tend to be more hampered in severe cases (461.6 vs 663.8 /uL; P = 0.027) when compared with non-severe group.

The function of CD4+, CD8+ T cells, and NK cells, as indicated by PMA/Ionomycin stimulated IFN-γ positive cells in these three subsets, was within normal range. And no significant difference was found between severe cases and non-severe ones.

We further analyzed different subsets of T cells. Both helper T cells (CD3+CD4+) and suppressor T cells (CD3+CD8+) in patients with COVID-19 were below normal levels, and the decline of helper T cells was more pronounced in severe cases (285.1 vs 420.5 /uL; P = 0.027). Similar tendency was also shown in the decline of suppressor T cells, although no

statistical difference between mild and severe cases yet (P = 0.197). The helper T and suppressor T ratio (Th/Ts) remained in the normal range, and showed no difference in the two subgroups. The percentage of naïve helper T cells (CD3+CD4+CD45RA+) increased (44.5 vs 35.0 %; P = 0.035) and memory helper T cells (CD3+CD4+CD45RO+) decreased (55.5 vs 65.0 %; P = 0.035) in severe cases when compare to non-severe cases. CD28 positive cytotoxic suppressor T cells (CD3+CD8+CD28+) percentage decreased in severe cases (54.5 vs 67.0 %; P = 0.035), while no significant difference was found in activated T cells (CD3+HLA-DR+) and activated suppressor T cells (CD3+CD8+HLA-DR+). Patients with COVID-19 presented lower level of regulatory T cells (CD3+CD4+CD25+CD127low+), and particularly obvious in severe cases (3.7 vs 4.5 /uL; P = 0.040). The decline of naïve (CD45RA+CD3+CD4+CD25+CD127low+) and induced regulatory T cells (CD45RO+CD3+CD4+CD25+CD127low+) had a more obvious trend in the severe group, although no significant difference.

Discussion

We reported here dysregulated immune system in a cohort of 452 patients with laboratory confirmed COVID-19 in Wuhan, China. Totally, increase of NLR, and T lymphopenia—in particular, decrease of CD4+ T cells—were common among patients with COVID-19, and more evident in the severe cases, but no significant change in the number of CD8+ cells and B cells. Based on these data, we suggested that COVID-19 might damage lymphocytes, especially T lymphocytes, and the immune system was impaired during the period of disease.

In the cohort, we observed that 44.0% of patients had at least one underlying disorder (i.e., hypertension, diabetes, chronic obstructive pulmonary disease), and a higher percentage of hypertension and cardiovascular disease in the severe cases than the mild individuals, in

consistent with those reports[5, 12], suggested that COVID-19 is more likely to infect those elder men with chronic comorbidities due to weaker immune functions.

In terms of laboratory tests, we noted that most of infected patients presented lymphopenia and elevated levels of infection-related biomarkers, More interestingly, a higher number of neutrophils and a lower number of lymphocytes, ie, the increase of neutrophil-to-lymphocyte ratio (NLR), were found in the severe group with COVID-19 compared to the mild group. NLR, a well-known marker of systemic inflammation and infection, has been studied as a predictor of bacterial infection, included pneumonia[13-15]. The increase of NLR in our study, consistent with the findings from Wang et al that several patients with COVID-19 had a rising neutrophil count and a falling lymphocyte count during the severe phase[12], indicated that serious disturbance in internal environment and potential critical condition in those severe infected cases.

Higher serum levels of pro-inflammatory cytokines (TNF-α, IL-1 and IL-6) and chemokines (IL-8) were found in patients with severe COVID-19 compared to individuals with mild disease, similar to the results in SARS and MERS[7, 16]. Cytokines and chemokines have been thought to play an important role in immunity and immunopathology during virus infections[16, 17]. Although there is no direct evidence for the involvement of pro-inflammatory cytokines and chemokines in lung pathology during COVID-19, the change of laboratory parameters, including elevated serum cytokine, chemokine levels, and increased NLR in infected patients were correlated with the severity of the disease and adverse outcome, suggesting a possible role for hyper-inflammatory responses in COVID-19 pathogenesis.

Virus-induced direct cytopathic effects and viral evasion of host immune responses are believed to play major roles in disease severity[16, 17]. A rapid and well-coordinated innate

immune response is the first line of defense against viral infections, however, when immune response is dysregulated, it will result in an excessive inflammation, even cause death[18]. In our study, we demonstrated pronounced lymphopenia and low counts of CD3+ cells and CD4+ cells in COVID-19 cases. The differentiation of naïve CD4+ T-cells into effector and memory subsets is one of the most fundamental facets of T-cell-mediated immunity[19]. And the balance between the naïve and memory CD4+ T cells is crucial for maintaining an efficient immune response. Our results of lymphocyte subsets with higher naïve CD4+ T-cell subpopulations and smaller percentages of memory cells, higher naïve: memory ratio, in severe cases indicated that immune system in severe infection subgroup was impaired more severely. In addition, the decrease of regulatory T cells, especially induced regulatory T cells which have a key role in restraining allergic inflammation at mucosal surfaces, was demonstrated in those infected patients, especially in the severe group. Furthermore, similar tendency was also presented in naïve regulatory T cells, which underlie the control of systemic and tissue specific autoimmunity. It has been shown that T cells, especially CD4+ and CD8+ T cells, play an important role in weakening or dampening overactive innate immune responses during viral infection [18]. Whereas, regulatory T cells, a subset of T helper cells, play a crucial role in negatively regulating the activation, proliferation, and effector functions of a wide range of immune cells for the maintenance of self-tolerance and immune homeostasis [20, 21]. Given that higher expression of proinflammatory cytokines and chemokines in COVID-19 patients, especially in the severe cases, the consumption of CD4+ and CD8+ T cells, and the decrease of regulatory T cells, presented in our study, might result in aggravated inflammatory responses, the production of cytokine storm and make damaged tissue worse. Although not so sure, correlative evidence from those severe patients with lower number of lymphocytes suggested a role for dysregulated immune responses in COVID-19 pathogenesis.

There were several limitations in our study which might make some potential bias. First, it was a retrospective, single center and small sample study of patients admitted to hospital; standardized data for a larger cohort would be better to assess the temporal change of immune response after infection with COVID-19. Second, those patients with COVID-19 who have bacterial co-infection or superinfection might affect the results of immune response. Most of them presented the increase of NLR and procalcitonin, and more evident in severe cases, indicated potential bacterial co-infection due to dysregulated immune system. Despite that, our study demonstrated several novel information about dysregulated immune response in COVID-19 patients that SARS-CoV-2 might mainly act on lymphocytes, especially T lymphocytes, induce a cytokine storm in the body, and generate a series of immune responses to damage the corresponding organs; thus, surveillance of NLR and lymphocyte subsets is helpful in the early screening of critical illness, diagnosis and treatment of COVID-19.

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None.

Declaration of interests

All authors declare no competing interests.

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Table 1: Demographics and baseline characteristics of patients with COVID-19

	No. (%)			
	All patients (n = 452)	Non-Severe (n = 166)	Severe (n = 286)	*P* value
Characteristics				
Age, Median (IQR), Range, years	58 (47-67), 22-95	53 (41.25-62), 22-92	61 (51-69), 26-95	<0.001
Sex				0.242
Male	235 (52.0%)	80 (48.2%)	155 (54.2%)	
Female	217 (48.0%)	86 (51.8%)	131 (45.8%)	
Smoking	7 (1.5%)	4 (2.4%)	3 (1.0%)	0.267
Chronic medical illness				
Any	201 (44.0%)	55 (33.1%)	146 (51.0%)	<0.001
Chronic obstructive pulmonary disease	12 (2.6%)	3 (1.8%)	9 (3.1%)	0.548
Hypertension	135 (29.5%)	30 (18.1)	105 (36.7%)	<0.001
Cardiovascular disease	27 (5.9%)	3 (1.8%)	24 (8.4%)	0.004
Cerebrovascular Disease	11 (2.4%)	3 (1.8%)	8 (2.8%)	0.753
Chronic liver disease	6 (1.3%)	3 (1.8%)	3 (1.0%)	0.674
Diabetes	75 (16.4%)	22 (13.3%)	53 (18.5%)	0.152
Tuberculosis	9 (19.7%)	2 (1.2%)	7 (2.4%)	0.496
Malignant tumour	14 (3.1%)	4 (2.4 %)	10 (3.5%)	0.587
Chronic kidney disease	10 (2.2%)	4 (2.4 %)	6 (2.1%)	1.000

Signs and symptoms				
Fever	423 (92.6%)	152 (91.6%)	271 (94.8%)	0.232
Dry cough	152 (33.3%)	56 (33.7%)	96 (33.6%)	1.000
Expectoration	189 (41.4%)	68 (41.0%)	121 (42.3%)	0.843
Hemoptysis	12 (2.6%)	2 (1.2%)	10 (3.5%)	0.225
Shortness of breath	232 (50.8)	65 (39.2%)	167 (58.4%)	<0.001
Myalgia	98 (21.4%)	32 (19.3%)	66 (23.1%)	0.407
Confusion	3 (0.7%)	0 (0.0%)	3 (1.0%)	0.301
Headache	52 (11.4%)	13 (7.8%)	39 (13.6%)	0.068
Dizziness	37 (8.1%)	9 (5.4%)	28 (9.8%)	0.112
Fatigue	212 (46.4%)	65 (39.2%)	147 (51.4%)	0.014
Rhinorrhoea	8 (1.8%)	2 (1.2%)	6 (2.1%)	0.716
Pharyngalgia	22 (4.8%)	10 (6.0%)	12 (4.2%)	0.376
Anorexia	96 (21.0%)	30 (18.1%)	66 (23.1%)	0.234
Nausea and vomiting	42 (9.2%)	10 (6.0%)	32 (11.2%)	0.092
Diarrhea	122 (26.7%)	44 (26.5%)	78 (27.3%)	0.913
Abdominal pain	23 (5.0%)	4 (2.4 %)	19 (6.6%)	0.073

Data are median (IQR), n (%), or n/N (%), where N is the total number of patients with available data. p values comparing Severe and non-Severe cases are from χ^2 test, Fisher's exact test, or Mann-Whitney U test. COVID-19= coronavirus disease 2019.

Table 2: Laboratory findings of patients with COVID-19

Laboratory Findings	Normal Range	Median (IQR)			*P* value
		All patients (n = 452)	Non-Severe (n = 166)	Severe (n = 286)	
Blood routine					
Leucocytes, × 10^9 per L	3.5–9.5	5.3 (3.9-7.5)	4.9 (3.7-6.1)	5.6 (4.3-8.4)	<0.001
Neutrophils, × 10^9 per L	1.8–6.3	3.9 (2.6-5.8)	3.2 (2.1-4.4)	4.3 (2.9-7.0)	<0.001
Neutrophil percentage, %	40.0-75.0	74.3 (64.3-83.9)	67.5 (57.8-75.8)	77.6 (68.9-86.5)	<0.001
Lymphocytes, × 10^9 per L;	1.1–3.2	0.9 (0.6-1.2)	1.0 (0.7-1.3)	0.8 (0.6-1.1)	<0.001
Lymphocyte percentage, %	20.0-50.0	17.5 (10.7-25.1)	21.4 (15.3-32.5)	14.1(8.8-21.4)	<0.001
Neutrophil-to-lymphocyte ratio		4.2 (2.5-7.7)	3.2 (1.8-4.9)	5.5 (3.3-10.0)	<0.001
Monocyte, × 10^9 per L	0.1–0.6	0.4 (0.3-0.5)	0.4 (0.3-0.5)	0.4 (0.3-0.5)	0.395
Monocyte percentage, %	3.0-10.0	7.1 (4.9-9.6)	8.4 (6.5-10.8)	6.6 (4.3-8.8)	<0.001
Eosinophils, × 10^9 per L	0.02-0.52	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	<0.001
Eosinophil percentage, %	0.4-8.0	0.0 (0.0-0.4)	0.2 (0.0-0.7)	0.0 (0.0-0.2)	<0.001
Basophils, × 10^9 per L	0.00-0.10	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.747
Basophil percentage, %	0.0-1.0	0.1 (0.1-0.2)	0.2 (0.0-0.3)	0.1 (0.0-0.2)	0.015
Infection-related biomarkers					
Procalcitonin, ng/mL	0.0–0.05	0.1 (0.0-0.2)	0.05 (0.03-0.09)	0.1 (0.0-0.2)	<0.001
Erythrocyte sedimentation rate,	0.0–15.0	31.5 (17.0-58.0)	28.0 (14.0-50.0)	34.0 (19.0-60.0)	0.123

mm/h					
Serum ferritin, ng/mL	15.0–150.0	662.4 (380.9-1311.9)	523.7 (299.1-840.4)	800.4 (452.9-1451.6)	<0.001
C-reactive protein, mg/L	0.0–1.0	44.1 (15.5-93.5)	33.2 (8.2-59.7)	57.9 (20.9-103.2)	<0.001
Inflammatory cytokines					
Tumor necrosis factor-α, pg/mL	0.0–8.1	8.6 (6.9-10.9)	8.4 (6.9-10.4)	8.7 (7.1-11.6)	0.037
Interleukin-1β, pg/mL	0.0–5.0	5.0 (5.0-5.0)	5.0 (5.0-5.0)	5.0 (5.0-5.0)	0.962
Interleukin-2R, U/mL	223.0–710.0	714.5 (514.5-1040.3)	663.5 (473.3-862.8)	757.0 (528.5-1136.3)	0.001
Interleukin-6, pg/mL	0.0–7.0	21.0 (6.1-47.2)	13.3 (3.9-41.1)	25.2 (9.5-54.5)	<0.001
Interleukin-8, pg/mL	0.0–62.0	16.7 (10.2-27.0)	13.7 (8.9-21.0)	18.4 (11.3-28.4)	<0.001
Interleukin-10, pg/mL	0.0–9.1	5.4 (5.0-9.7)	5.0 (5.0-7.0)	6.6 (5.0-11.3)	<0.001
Immunoglobulins					
Immunoglobulin A	0.82-4.53	2.21 (1.65-2.79)	2.14 (1.66-2.71)	2.26 (1.57-2.89)	0.285
Immunoglobulin G	7.51-15.60	11.75 (9.70-13.60)	11.85 (10.13-13.40)	11.7 (9.53-13.8)	0.551
Immunoglobulin M	0.46-3.04	0.95 (0.70-1.31)	1.02 (0.77-1.37)	0.90 (0.69-1.28)	0.033
Complement proteins					
C3	0.65-1.39	0.88 (0.77-1.00)	0.88 (0.77-1.00)	0.89 (0.77-1.00)	0.942
C4	0.16-0.38	0.26 (0.20-0.31)	0.26 (0.20-0.31)	0.26 (0.20-0.31)	0.851

Data are median (IQR). p values comparing Severe and non-Severe cases are from χ^2 test, Fisher's exact test, or Mann-Whitney U test. COVID-19= coronavirus disease 2019.

Table 3: Lymphocyte subset analysis in patients with COVID-19

	Normal Range	Mean (SD)			*P* value
		All patients (n =44)	Non-Severe (n = 17)	Severe (n = 27)	
Lymphocyte Subsets					
T cells+B cells+NK cells /ul	1100.0-3200.0	852.9 (412.0)	1020.1 (396.5)	743.6 (384.4)	0.032
T cells+B cells+NK cells %	95.0-105.0	98.9 (1.0)	99.2 (0.6)	98.6 (1.2)	0.103
B cells (CD3-CD19+) /ul	90.0-560.0	179.7 (143.1)	196.1 (144.9)	169.0 (140.9)	0.559
B cells (CD3-CD19+) %	5.0-18.0	20.5 (10.9)	18.5 (8.1)	21.8 (12.2)	0.353
T cells (CD3+CD19-) /ul	955.0-2860.0	541.5 (292.7)	663.8 (291.3)	461.6 (264.7)	0.027
T cells (CD3+CD19-) %	50.0-84.0	61.3 (10.1)	63.4 (8.5)	60.0 (10.8)	0.283
NK cells (CD3-/CD16+CD56+) /ul	150.0-1100.0	131.7 (83.1)	160.2 (90.8)	113.0 (71.8)	0.072
NK cells (CD3-/CD16+CD56+) %	7.0-40.0	17.0 (10.1)	17.2 (10.1)	16.9 (10.1)	0.926
Lymphocyte function					
IFN-γ+ CD4+ T cells /Th %	14.54-36.96	21.2 (12.2)	22.6 (10.2)	20.2 (13.3)	0.557
IFN-γ+ CD8+ T cells /Ts %	34.93-87.95	48.6 (13.7)	46.9 (11.6)	49.7 (14.8)	0.541
IFN-γ+ NK cells /NK %	61.2-92.65	68.0 (14.7)	66.7 (19.3)	68.8 (10.5)	0.677
T cells Subsets					
Th cells (CD3+CD4+) /ul	550.0-1440.0	338.6 (196.3)	420.5 (207.8)	285.1 (168.0)	0.027
Th cells (CD3+CD4+) %	27.0-51.0	38.3 (8.1)	39.8 (7.5)	37.2 (8.4)	0.314
Ts cells (CD3+CD8+) /ul	320.0-1250.0	173.4 (115.2)	201.9 (107.1)	154.7 (116.5)	0.197

Ts cells (CD3+CD8+) %	15.0-44.0	19.6 (8.1)	19.5 (6.2)	19.7 (9.2)	0.930
Th/Ts	0.71-2.78	2.4 (1.2)	2.2 (0.6)	2.5 (1.5)	0.415
Naïve Th cells (CD3+CD4+CD45RA+)/Th %	29.41-55.41	40.7 (13.3)	35.0 (13.0)	44.5 (12.2)	0.035
Memory Th cells (CD3+CD4+CD45RO+)/Th %	44.44-68.94	59.3 (13.3)	65.0 (13.0)	55.5 (12.2)	0.035
CD28+ Th cells (CD3+CD4+CD28+)/Th %	84.11-100.00	90.0 (14.0)	91.2 (12.7)	90.6 (14.7)	0.911
CD28+ Ts cells (CD3+CD8+CD28+)/Ts %	48.04-77.14	59.6 (17.7)	67.0 (16.0)	54.5 (16.9)	0.035
Activated T cells (CD3+HLA-DR+) /ul	9.04-25.62	15.0 (5.8)	14.4 (5.2)	15.4 (6.2)	0.636
Activated Ts cells (CD3+CD8+HLA-DR+)/Ts %	20.73-60.23	39.8 (10.7)	36.3 (10.7)	42.2 (10.1)	0.109
Regulatory T cells (CD3+CD4+CD25+CD127low+) /ul	5.36-6.30	4.1 (1.2)	4.5 (0.9)	3.7 (1.3)	0.040
Naïve regulatory T cells (CD45RA+CD3+CD4+CD25+CD127low+) /ul	2.07-4.55	1.0 (0.5)	1.1 (0.5)	0.9 (0.5)	0.502
Induced regulatory T cells (CD45RO+CD3+CD4+CD25+CD127low+) /ul	1.44-2.76	3.1 (1.1)	3.5 (0.8)	1.8 (1.2)	0.064

Data are mean (SD). p values comparing Severe and non-Severe cases are from T test, or Mann-Whitney U test. COVID-19 = coronavirus disease 2019, NK cells = natural killer cells, Th cells = helper T cells, Ts cells = suppressor T cells.