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Dysregulation of Immune Response in Patients With Coronavirus 2019 (COVID-19) in Wuhan, China

Chuan Qin,[1,a] Luoqi Zhou,[1,a] Ziwei Hu,[1] Shuoqi Zhang,[2] Sheng Yang,[1] Yu Tao MD,[3] Cuihong Xie,[4] Ke Ma,[5] Ke Shang,[1] Wei Wang,[1] and Dai-Shi Tian[1]

[1]Department of Neurology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, [2]Department of Radiology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, [3]Department of Respiratory and Critical Care Medicine, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, [4]Department of Emergency Medicine, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, and [5]Department of Infectious Diseases, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

Background. In December 2019, coronavirus 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 10 January to 12 February 2020 were collected and analyzed. The data on laboratory examinations, including peripheral lymphocyte subsets, were analyzed and compared between patients with severe and nonsevere infection.

Results. Of the 452 patients with COVID-19 recruited, 286 were diagnosed as having 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 lymphocyte counts, higher leukocyte counts and neutrophil-lymphocyte ratio (NLR), as well as lower percentages of monocytes, eosinophils, and basophils. Most severe cases demonstrated elevated levels of infection-related biomarkers and inflammatory cytokines. The number of T cells significantly decreased, and were more impaired in severe cases. Both helper T (Th) cells and suppressor T cells in patients with COVID-19 were below normal levels, with lower levels of Th cells in the severe group. The percentage of naive Th cells increased and memory Th cells decreased in severe cases. Patients with COVID-19 also have lower levels of regulatory T cells, which are more obviously decreased 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.

The outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which first emerged in Wuhan in December 2019, has rapidly spread throughout China in the past 2 months [1, 2]. Considering the ongoing outbreak in China and fast worldwide spread of SARS-Cov-2 caused coronavirus 2019 (COVID-19), it has led to the declaration of a Public Health Emergency of International Concern by the World Health Organization (WHO) on 30 January 2020 [3]. As of 16 February 2020, a total of 58 182 laboratory-confirmed cases have 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 COVID-19 was more likely to occur in older men with comorbidities [1, 4, 5], 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 yet been completely understood. Several studies have shown that increased amounts of proinflammatory cytokines in serum were associated with pulmonary inflammation and extensive lung damage in SARS [6] and middle east respiratory syndrome coronavirus (MERS-CoV) infection [7], and recently in COVID-19 [1]. 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 nonsevere cases.

METHODS

Study Design and Participants

We retrospectively recruited a total of 452 patients with COVID-19 from 10 January to 12 February 2020 at Tongji Hospital, the largest comprehensive medical treatment center of 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 (Institutional Review Board ID: TJ-C20200121). Written informed consent was waived by

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[a]C. Q. and L. Z. contributed equally to this work.

Correspondence: D.-S. Tian, Department of Neurology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, P.R. China (tiands@tjh.tjmu.edu.cn or tiandaishi@126.com).

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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 [8]. Those who met the following criteria were defined as having severe-type infection: (1) respiratory distress with a respiratory rate over 30 breaths per minute, (2) oxygen saturation ≤93% in the resting state, and (3) arterial blood oxygen partial pressure (PaO_2) /oxygen concentration (FiO_2) ≤300 mm Hg.

Data Collection

Data including demographic data, medical history, symptoms, signs, and laboratory findings were collected from patients' medical records. 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 subset percentage were analyzed with a FACSCanto flow cytometer (BD, Franklin Lakes, USA) for those patients with COVID-19 on admission [9]. The absolute numbers of different lymphocyte subsets were calculated by multiplying the percentages with total lymphocyte count. Phorbol 12-Myristate 13-Acetate (PMA)/ionomycin-stimulated lymphocyte function assay was performed as described previously [10]. The percentages of interferon-γ (IFN-γ)–positive cells in different cell subsets were defined as the active parts of these immune cells. The data were reviewed by a trained team of physicians in Tongji Hospital.

Real-time Reverse Transcriptase–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 a PAN9600 Automated Nucleic Acid Extraction System (Tianlong, Xi'an, China), according to the manufacturer's instructions, followed by 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 thermus aquaticus (Taq) DNA polymerase, and 5 μL nucleic acid. Conditions for the amplifications include reverse transcription at 50°C for 30 minutes, predenaturation at 95°C for 10 minutes, followed by 5 cycles of 94°C for 15 seconds, 50°C for 30 seconds and 72°C for 30 seconds, and 40 cycles of 94°C for 10 seconds and 58°C for 30 seconds for fluorescence 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 describe the categorical variables as frequency rates and percentages and continuous variables as means and SDs, medians and interquartile ranges (IQRs). 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 χ^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 .05 were considered statistically significant.

RESULTS

Demographic and Clinical Characteristics of Patients With COVID-19

By 12 February 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 having severe infection. Demographic and clinical characteristics of the 452 patients with COVID-19 was shown in Table 1. In total, the median age was 58 years (IQR, 47–67; range, 22–95 years) and 235 (52.0%) were men. Compared with patients with nonsevere infection, patients with severe infection were significantly older (median age, 61 [IQR, 51–69] years vs 53 [IQR, 41–62] years; $P < .001$). The proportion of men in the severe group (54.2% men) were not significantly different from the nonsevere group. Of the 452 patients with COVID-19, 201 (44.0%) patients had chronic diseases (ie, hypertension, diabetes, chronic obstructive pulmonary disease), and a higher percentage in the severe cases (146 [51.0%]) than in the mild cases (55 [33.1%]). And those patients with severe infection were significantly more likely to have concomitant hypertension and cardiovascular diseases (36.7% vs 18.1%; $P < .001$; and 8.4% vs 1.8%; $P = .004$; 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, patients with severe infection were significantly more likely to have shortness of breath and fatigue (58.4% vs 39.2%; $P < .001$; and 51.4% vs 39.2%; $P = .014$; respectively) than patients with nonsevere infection.

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 (ie, procalcitonin, erythrocyte sedimentation rate, serum ferritin, and C-reactive protein), and several elevated inflammatory cytokines (ie, tumor necrosis factor-α [TNF-α], interleukin [IL]-2R and IL-6), and there were numerous differences in blood cell counts and infection-related biomarkers between the severe group and the nonsevere group. Severe cases had higher leukocyte (5.6 vs 4.9×10^9; $P < .001$) and neutrophil (4.3 vs 3.2×10^9; $P < .001$) counts, lower lymphocytes counts (0.8 vs 1.0×10^9; $P < .001$), a higher neutrophil-to-lymphocyte ratio (NLR;

Table 1. Demographic and Baseline Characteristics of Patients With COVID-19

	All Patients (N = 452)	Nonsevere (n = 166)	Severe (n = 286)	*P*
Characteristics				
Age, median (IQR), range, y	58 (47–67), 22–95	53 (41.25–62), 22–92	61 (51–69), 26–95	<.001
Sex				.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)	.267
Chronic medical illness				
Any	201 (44.0)	55 (33.1)	146 (51.0)	<.001
Chronic obstructive pulmonary disease	12 (2.6)	3 (1.8)	9 (3.1)	.548
Hypertension	135 (29.5)	30 (18.1)	105 (36.7)	<.001
Cardiovascular disease	27 (5.9)	3 (1.8)	24 (8.4)	.004
Cerebrovascular disease	11 (2.4)	3 (1.8)	8 (2.8)	.753
Chronic liver disease	6 (1.3)	3 (1.8)	3 (1.0)	.674
Diabetes	75 (16.4)	22 (13.3)	53 (18.5)	.152
Tuberculosis	9 (19.7)	2 (1.2)	7 (2.4)	.496
Malignant tumor	14 (3.1)	4 (2.4)	10 (3.5)	.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)	.232
Dry cough	152 (33.3)	56 (33.7)	96 (33.6)	1.000
Expectoration	189 (41.4)	68 (41.0)	121 (42.3)	.843
Hemoptysis	12 (2.6)	2 (1.2)	10 (3.5)	.225
Shortness of breath	232 (50.8)	65 (39.2)	167 (58.4)	<.001
Myalgia	98 (21.4)	32 (19.3)	66 (23.1)	.407
Confusion	3 (0.7)	0 (0.0)	3 (1.0)	.301
Headache	52 (11.4)	13 (7.8)	39 (13.6)	.068
Dizziness	37 (8.1)	9 (5.4)	28 (9.8)	.112
Fatigue	212 (46.4)	65 (39.2)	147 (51.4)	.014
Rhinorrhea	8 (1.8)	2 (1.2)	6 (2.1)	.716
Pharyngalgia	22 (4.8)	10 (6.0)	12 (4.2)	.376
Anorexia	96 (21.0)	30 (18.1)	66 (23.1)	.234
Nausea and vomiting	42 (9.2)	10 (6.0)	32 (11.2)	.092
Diarrhea	122 (26.7)	44 (26.5)	78 (27.3)	.913
Abdominal pain	23 (5.0)	4 (2.4)	19 (6.6)	.073

Data are median (IQR), n (%), in which N is the total number of patients with available data. *P* values comparing severe and nonsevere cases are derived from χ^2 test, Fisher' exact test, or Mann-Whitney *U* test.
Abbreviations: COVID-19, coronavirus 2019; IQR, interquartile range.

5.5 vs 3.2; $P < .001$), as well as lower percentages of monocytes (6.6 vs 8.4 %; $P < .001$), eosinophils (0.0 vs 0.2%; $P < .001$), and basophils (0.1 vs 0.2%; $P = .015$). Compared with the nonsevere group, most of severe cases demonstrated elevated levels of infection-related biomarkers, including procalcitonin (0.1 vs 0.05 ng/mL; $P < .001$), serum ferritin (800.4 vs 523.7 ng/mL; $P < .001$), and C-reactive protein (57.9 vs 33.2 mg/L; $P < .001$). Several inflammatory cytokines were also elevated in severe cases compared with the nonsevere cases, including IL-2R (757.0 vs 663.5 U/mL; $P = .001$), IL-6 (25.2 vs 13.3 pg/mL; $P < .001$), IL-8 (18.4 vs 13.7 pg/mL; $P < .001$), IL-10 (6.6 vs 5.0 pg/mL; $P < .001$), and TNF-α (8.7 vs 8.4 pg/mL; $P = .037$). Immunoglobulins (IgA, IgG, and IgM) and complement proteins (C3 and C4) in patients with COVID-19 were within the 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 was slightly decreased in severe cases.

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 natural killer (NK) cells were significantly decreased in patients with COVID-19 (852.9/μL), which was more evident in the severe cases (743.6 vs 1020.1/μL; $P = .032$) compared with the nonsevere group. The mean values of the 3 main subsets of lymphocytes were generally decreased in patients with COVID-19, as T cells and NK cells were below normal levels and B cells were 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 tended to be more impaired in severe cases (461.6 vs 663.8/μL; $P = .027$) when compared with the nonsevere group.

The function of CD4+, CD8+ T cells, and NK cells, as indicated by PMA/ionomycin-stimulated IFN-γ–positive cells in

Table 2. Laboratory Findings of Patients With COVID-19

Laboratory Findings	Normal Range	All Patients (N = 452)	Nonsevere (n = 166)	Severe (n = 286)	*P*
Blood routine					
Leucocytes, $\times 10^9$/L	3.5–9.5	5.3 (3.9–7.5)	4.9 (3.7–6.1)	5.6 (4.3–8.4)	<.001
Neutrophils, $\times 10^9$/L	1.8–6.3	3.9 (2.6–5.8)	3.2 (2.1–4.4)	4.3 (2.9–7.0)	<.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)	<.001
Lymphocytes, $\times 10^9$/L	1.1–3.2	0.9 (0.6–1.2)	1.0 (0.7–1.3)	0.8 (0.6–1.1)	<.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)	<.001
Neutrophil-to-lymphocyte ratio	…	4.2 (2.5–7.7)	3.2 (1.8–4.9)	5.5 (3.3–10.0)	<.001
Monocytes, $\times 10^9$/L	0.1–0.6	0.4 (0.3–0.5)	0.4 (0.3–0.5)	0.4 (0.3–0.5)	.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)	<.001
Eosinophils, $\times 10^9$/L	0.02–0.52	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)	<.001
Basophils, $\times 10^9$/L	0.00–0.10	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)	.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)	<.001
Erythrocyte sedimentation rate, mm/h	0.0–15.0	31.5 (17.0–58.0)	28.0 (14.0–50.0)	34.0 (19.0–60.0)	.123
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)	<.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)	<.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)	.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)	.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)	.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)	<.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)	<.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)	<.001
Immunoglobulins					
Immunoglobulin A, g/L	0.82–4.53	2.21 (1.65–2.79)	2.14 (1.66–2.71)	2.26 (1.57–2.89)	.285
Immunoglobulin G, g/L	7.51–15.60	11.75 (9.70–13.60)	11.85 (10.13–13.40)	11.7 (9.53–13.8)	.551
Immunoglobulin M, g/L	0.46–3.04	0.95 (0.70–1.31)	1.02 (0.77–1.37)	0.90 (0.69–1.28)	.033
Complement proteins					
C3, g/L	0.65–1.39	0.88 (0.77–1.00)	0.88 (0.77–1.00)	0.89 (0.77–1.00)	.942
C4, g/L	0.16–0.38	0.26 (0.20–0.31)	0.26 (0.20–0.31)	0.26 (0.20–0.31)	.851

Data are median (IQR). *P* values comparing severe and nonsevere cases are derived from χ^2 test, Fisher' exact test, or Mann-Whitney *U* test.
Abbreviations: COVID-19, coronavirus 2019; IQR, interquartile range.

Table 3. Lymphocyte Subset Analysis in Patients With COVID-19

		Normal Range	All Patients (N = 44)	Nonsevere (n = 17)	Severe (n = 27)	*P*
Lymphocyte subsets						
	T cells + B cells + NK cells/μL	1100.0–3200.0	852.9 (412.0)	1020.1 (396.5)	743.6 (384.4)	.032
	T cells + B cells + NK cells, %	95.0–105.0	98.9 (1.0)	99.2 (0.6)	98.6 (1.2)	.103
	B cells (CD3– CD19+)/μL	90.0–560.0	179.7 (143.1)	196.1 (144.9)	169.0 (140.9)	.559
	B cells (CD3– CD19+), %	5.0–18.0	20.5 (10.9)	18.5 (8.1)	21.8 (12.2)	.353
	T cells (CD3+ CD19–)/μL	955.0–2860.0	541.5 (292.7)	663.8 (291.3)	461.6 (264.7)	.027
	T cells (CD3+ CD19–), %	50.0–84.0	61.3 (10.1)	63.4 (8.5)	60.0 (10.8)	.283
	NK cells (CD3–/CD16+ CD56+)/μL	150.0–1100.0	131.7 (83.1)	160.2 (90.8)	113.0 (71.8)	.072
	NK cells (CD3–/CD16+ CD56+), %	7.0–40.0	17.0 (10.1)	17.2 (10.1)	16.9 (10.1)	.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)	.557
	IFN-γ+ CD8+ T cells/Ts, %	34.93–87.95	48.6 (13.7)	46.9 (11.6)	49.7 (14.8)	.541
	IFN-γ+ NK cells/NK, %	61.2–92.65	68.0 (14.7)	66.7 (19.3)	68.8 (10.5)	.677
T-cell subsets						
	Th cells (CD3+ CD4+)/μL	550.0–1440.0	338.6 (196.3)	420.5 (207.8)	285.1 (168.0)	.027
	Th cells (CD3+ CD4+), %	27.0–51.0	38.3 (8.1)	39.8 (7.5)	37.2 (8.4)	.314
	Ts cells (CD3+ CD8+)/μL	320.0–1250.0	173.4 (115.2)	201.9 (107.1)	154.7 (116.5)	.197
	Ts cells (CD3+ CD8+), %	15.0–44.0	19.6 (8.1)	19.5 (6.2)	19.7 (9.2)	.930
	Th/Ts	0.71–2.78	2.4 (1.2)	2.2 (0.6)	2.5 (1.5)	.415
	Naive Th cells (CD3+ CD4+ CD45RA+)/Th, %	29.41–55.41	40.7 (13.3)	35.0 (13.0)	44.5 (12.2)	.035
	Memory Th cells (CD3+ CD4+ CD45RO+)/Th %	44.44–68.94	59.3 (13.3)	65.0 (13.0)	55.5 (12.2)	.035
	CD28 + Th cells (CD3+ CD4+ CD28+)/Th, %	84.11–100.00	90.0 (14.0)	91.2 (12.7)	90.6 (14.7)	.911
	CD28 + Ts cells (CD3+ CD8+ CD28+)/Ts, %	48.04–77.14	59.6 (17.7)	67.0 (16.0)	54.5 (16.9)	.035
	Activated T cells (CD3+ HLA-DR+)/μL	9.04–25.62	15.0 (5.8)	14.4 (5.2)	15.4 (6.2)	.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)	.109
	Regulatory T cells (CD3+ CD4+ CD25+ CD127low+)/μL	5.36–6.30	4.1 (1.2)	4.5 (.9)	3.7 (1.3)	.040
	Naive regulatory T cells (CD45RA+ CD3+ CD4+ CD25 + CD127low+)/μL	2.07–4.55	1.0 (0.5)	1.1 (0.5)	0.9 (0.5)	.502
	Induced regulatory T cells (CD45RO+ CD3+ CD4+ CD25+ CD127low+)/μL	1.44–2.76	3.1 (1.1)	3.5 (0.8)	1.8 (1.2)	.064

Data are mean (SD). *P* values comparing severe and nonsevere cases are derived from *t* test or Mann-Whitney *U* test.
Abbreviations: COVID-19, coronavirus 2019; IFN-γ, interferon-γ; NK, natural killer; Th, helper T; Ts, suppressor T.

these 3 subsets, was within the normal range. No significant differences were found between severe cases and nonsevere cases.

We further analyzed different subsets of T cells. Both helper T (Th) cells (CD3+, CD4+) and suppressor T cells (CD3+, CD8+) in patients with COVID-19 were below normal levels, and the decline in Th cells was more pronounced in severe cases (285.1 vs 420.5/μL; $P = .027$). A similar tendency was also shown in the decline in suppressor T cells, although there was no statistical difference between mild and severe cases ($P = .197$). The Th and suppressor T ratio (Th/Ts) remained in the normal range, and showed no difference between the 2 subgroups. The percentage of naive Th cells (CD3+, CD4+, CD45RA+) increased (44.5 vs 35.0 %; $P = .035$) and memory Th cells (CD3+, CD4+, CD45RO+) decreased (55.5 vs 65.0 %; $P = .035$) in severe cases when compared with nonsevere cases. CD28-positive cytotoxic suppressor T cells (CD3+, CD8+, CD28+) percentage decreased in severe cases (54.5 vs 67.0 %; $P = .035$), 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 levels of regulatory T cells (CD3+, CD4+, CD25+, CD127low+), which was particularly obvious in severe cases (3.7 vs 4.5/μL; $P = .040$). The decline in naive (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 there was no significant difference.

DISCUSSION

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

In the cohort, we observed that 44.0% of patients had at least 1 underlying disorder (ie, hypertension, diabetes, chronic obstructive pulmonary disease), and a higher percentage of hypertension and cardiovascular disease in the severe cases than in the mild cases, which is consistent with reports [1, 11] that suggested that COVID-19 is more likely to infect elderly men with chronic comorbidities due to weaker immune functions.

In terms of laboratory tests, we noted that most of the infected patients presented with lymphopenia and elevated levels of infection-related biomarkers, More interestingly, a higher number of neutrophils and a lower number of lymphocytes (ie, the increase in NLR) were found in the severe group with COVID-19 compared with the mild group. NLR, a well-known marker of systemic inflammation and infection, has been studied as a predictor of bacterial infection, included pneumonia [12–14]. The increase in NLR in our study, consistent with the findings from Wang et al [11] that several patients with COVID-19 had an increased neutrophil count and a decreased lymphocyte count during the severe phase, indicated the potential critical condition and serious disturbance of internal environment in those severe infected cases.

Higher serum levels of proinflammatory cytokines (TNF-α, IL-1, and IL-6) and chemokines (IL-8) were found in patients with severe COVID-19 compared with individuals with mild disease, similar to the results in SARS and MERS [6, 15]. Cytokines and chemokines have been thought to play an important role in immunity and immunopathology during viral infections [15, 16]. Although there is no direct evidence for the involvement of proinflammatory cytokines and chemokines in lung pathology during COVID-19, the changes in 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 hyperinflammatory 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 [15, 16]. A rapid and well-coordinated innate immune response is the first line of defense against viral infections; however, when the immune response is dysregulated, it will result in excessive inflammation, and even cause death [17]. In our study, we demonstrated pronounced lymphopenia and low counts of CD3+ cells and CD4+ cells in COVID-19 cases. The differentiation of naive CD4+ T cells into effector and memory subsets is one of the most fundamental facets of T-cell–mediated immunity [18]. And the balance between the naive and memory CD4+ T cells is crucial for maintaining an efficient immune response. Our results of lymphocyte subsets with higher naive CD4+ T-cell subpopulations and smaller percentages of memory cells and a higher naive-to-memory CD4+ T-cell ratio in severe cases indicated that the immune system in the severe infection subgroup was impaired more severely. In addition, the decrease in 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, a similar tendency was also present in naive 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 [17], although regulatory T cells, a subset of Th 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 [19, 20]. Given the higher expression of proinflammatory cytokines and chemokines in patients with COVID-19, especially in the severe cases, the consumption of CD4+ and CD8+ T cells, and the decrease in regulatory T cells, presented in our study might result in aggravated inflammatory responses and the production of a cytokine storm, and worsen damaged tissue. Although not conclusive, correlative evidence from those patients with severe infection with a lower number of lymphocytes suggested a role for dysregulated immune responses in COVID-19 pathogenesis.

There were several limitations to our study that might cause some potential bias. First, it was a retrospective, single-center, small-sample study of patients admitted to the hospital; standardized data for a larger cohort would be better to assess the temporal change in immune response after infection with COVID-19. Second, co-infection with bacteria or superinfection might affect the results of the immune response in those patients with COVID-19. Most of them presented with an increase in NLR and procalcitonin, which was more evident in severe cases, and indicated potential bacterial coinfection due to a dysregulated immune system. Despite that, our study demonstrated several novel findings on dysregulated immune response in patients with COVID-19 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 and diagnosis and treatment of COVID-19.

Note

Potential conflicts of interest. The authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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