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*J Clin Invest.* 2020;130(5):2620-2629. <https://doi.org/10.1172/JCI137244>.

Clinical Medicine

COVID-19

Immunology

Infectious disease

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In this retrospective study, the clinical and immunological characteristics of 21 patients (17 male and 4 female) with COVID-19 were analyzed. These patients were classified as severe (11 cases) and moderate (10 cases) according to the guidelines released by the National Health Commission of China.

The median age of severe and moderate cases was 61.0 and 52.0 years, respectively. Common clinical manifestations included fever, cough, and fatigue. Compared with moderate cases, severe cases more frequently had dyspnea, lymphopenia, and hypoalbuminemia, with higher levels of alanine aminotransferase, lactate dehydrogenase, C-reactive protein, ferritin, and D-dimer as well as markedly higher levels of IL-2R, IL-6, IL-10, and TNF- $\alpha$ . Absolute numbers of T lymphocytes, CD4<sup>+</sup> T cells, and CD8<sup>+</sup> T cells decreased in nearly all the patients, and were markedly lower in severe cases (294.0, 177.5, and 89.0  $\times 10^6/L$ , respectively) than moderate cases (640.5, 381.5, and 254.0  $\times 10^6/L$ , [...])

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# Clinical and immunological features of severe and moderate coronavirus disease 2019

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**BACKGROUND.** Since December 2019, an outbreak of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in Wuhan, and is now becoming a global threat. We aimed to delineate and compare the immunological features of severe and moderate COVID-19.

**METHODS.** In this retrospective study, the clinical and immunological characteristics of 21 patients (17 male and 4 female) with COVID-19 were analyzed. These patients were classified as severe (11 cases) and moderate (10 cases) according to the guidelines released by the National Health Commission of China.

**RESULTS.** The median age of severe and moderate cases was 61.0 and 52.0 years, respectively. Common clinical manifestations included fever, cough, and fatigue. Compared with moderate cases, severe cases more frequently had dyspnea, lymphopenia, and hypoalbuminemia, with higher levels of alanine aminotransferase, lactate dehydrogenase, C-reactive protein, ferritin, and D-dimer as well as markedly higher levels of IL-2R, IL-6, IL-10, and TNF- $\alpha$ . Absolute numbers of T lymphocytes, CD4<sup>+</sup> T cells, and CD8<sup>+</sup> T cells decreased in nearly all the patients, and were markedly lower in severe cases (294.0, 177.5, and 89.0  $\times 10^6/L$ , respectively) than moderate cases (640.5, 381.5, and 254.0  $\times 10^6/L$ , respectively). The expression of IFN- $\gamma$  by CD4<sup>+</sup> T cells tended to be lower in severe cases (14.1%) than in moderate cases (22.8%).

**CONCLUSION.** The SARS-CoV-2 infection may affect primarily T lymphocytes, particularly CD4<sup>+</sup> and CD8<sup>+</sup> T cells, resulting in a decrease in numbers as well as IFN- $\gamma$  production by CD4<sup>+</sup> T cells. These potential immunological markers may be of importance because of their correlation with disease severity in COVID-19.

**TRIAL REGISTRATION.** This is a retrospective observational study without a trial registration number.

**FUNDING.** This work is funded by grants from Tongji Hospital for the Pilot Scheme Project, and partly supported by the Chinese National Thirteenth Five Years Project in Science and Technology for Infectious Disease (2017ZX10202201).

## Introduction

Coronaviruses (CoVs) are a large family of respiratory viruses that can cause diseases ranging from the common cold to the Middle East respiratory syndrome (MERS) and the severe acute respiratory syndrome (SARS) (1, 2), both of which are zoonotic in origin and induce fatal lower respiratory tract infection as well as extrapulmonary manifestations. The new CoV, officially designated as the

SARS coronavirus 2 (SARS-CoV-2), is a member of  $\beta$ -CoV lineage B, which was first identified in Wuhan by the Chinese Center for Disease Control and Prevention (CDC) (3, 4). Recent reports have provided evidence for person-to-person transmission of SARS-CoV-2 in family and hospital settings (5, 6). As of February 27, 2020, the number of SARS-CoV-2 cases globally had eclipsed 82,567, largely exceeding the total number of SARS cases during the 2003 epidemic, and more than 2810 people have now died. The outbreak of SARS-CoV-2-induced coronavirus disease 2019 (COVID-19) has put health authorities on high alert in China and across the globe.

It has been revealed that SARS-CoV-2 has a genome sequence 75% to 80% identical to the SARS-CoV and has more similarities to several bat CoVs (7). Both clinical and epidemiological features of patients with COVID-19 demonstrate that the SARS-CoV-2 infection can cause clusters of severe respiratory illness with clinical presentations greatly resembling SARS-CoV, leading to intensive care unit (ICU) admission and high mortality (8). Clinical manifestations have included fever, fatigue, dry cough, shortness

### ► Related Commentary: p. 2202

**Authorship note:** GC, DW, WG, and YC are co-first authors. DH, HW, TW, and XZ are co-second authors.

**Conflict of interest:** The authors have declared that no conflict of interest exists.

**Role of funding source:** The funding source supports the studies of infectious disease, including emerging infectious diseases.

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**Submitted:** February 12, 2020; **Accepted:** March 27, 2020; **Published:** March 27, 2020.

**Reference information:** *J Clin Invest.* 2020;130(5):2620–2629.

<https://doi.org/10.1172/JCI137244>.

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

	All patients (n = 21)	Severe cases (n = 11)	Moderate cases (n = 10)	P value
<b>Characteristics</b>				
Males, n (%)	17 (81.0%)	10 (90.9%)	7 (70.0%)	0.31
Age, yrs	56.0 (50.0–65.0)	61.0 (56.5–66.0)	52.0 (42.8–56.0)	0.043
>50	15 (71.4%)	10 (90.9%)	5 (50.0%)	0.043
Huanan Seafood Market exposure, n (%)	4 (19.0%)	1 (9.1%)	3 (30.0%)	0.31
Any comorbidity, n (%)	7 (33.3%)	5 (45.5%)	2 (20.0%)	0.36
Hypertension, n (%)	5 (23.8%)	4 (36.4%)	1 (10.0%)	0.31
Diabetes, n (%)	3 (14.3%)	2 (18.2%)	1 (10.0%)	1.00
<b>Signs and symptoms</b>				
Fever, n/N (%)	20/20 (100%)	10/10 (100%)	10/10 (100%)	NA
Highest temperature, °C	38.7 (38.5–39.1)	38.6 (38.4–39.3)	38.8 (38.6–39.0)	0.87
38.1°C–39.0°C, n/N (%)	12/19 (63.2%)	5/9 (55.6%)	7/10 (70.0%)	0.52
>39.0°C, n/N (%)	7/19 (36.8%)	4/9 (44.4%)	3/10 (30.0%)	
Cough, n/N (%)	16/20 (80.0%)	7/10 (70.0%)	9/10 (90.0%)	0.58
Fatigue, n/N (%)	17/20 (85.0%)	10/10 (100.0%)	7/10 (70.0%)	0.21
Myalgia, n/N (%)	8/20 (40.0%)	5/10 (50.0%)	3/10 (30.0%)	0.65
Sputum production, n/N (%)	5/20 (25%)	2/10 (20.0%)	3/10 (30.0%)	1.00
Headache, n/N (%)	2/20 (10.0%)	1/10 (10.0%)	1/10 (10.0%)	1.00
Diarrhea, n/N (%)	4/20 (20.0%)	1/10 (10.0%)	3/10 (30.0%)	0.58
Chest tightness, n/N (%)	11/20 (55.0%)	8/10 (80.0%)	3/10 (30.0%)	0.07
Coma, n (%)	1 (4.8%)	1 (9.1%)	0 (0.0%)	1.00
Dyspnea, n (%)	11 (52.4%)	11 (100.0%)	0 (0.0%)	0.000
Days from illness onset to dyspnea	8.0 (7.0–10.0)	8.0 (7.0–10.0)	NA	NA
Systolic pressure, mmHg	122.0 (109.0–135.0)	124.0 (118.5–145.5)	120.0 (107.5–134.0)	0.17
>140 mmHg, n (%)	4 (19.0%)	4 (36.4%)	0 (0.0%)	0.09
Heart rate, bpm	89.0 (78.0–106.0)	95.0 (77.0–108.0)	89.0 (85.5–96.0)	0.90
Respiratory rate, per min	21.0 (20.0–25.0)	25.0 (22.5–31.0)	20.0 (20.0–20.8)	0.005
≥30, n (%)	5 (23.8%)	5 (45.5%)	0 (0.0%)	0.035
SpO <sub>2</sub> ≤ 93% on room air, n (%)	11 (52.4%)	11 (100.0%)	0 (0.0%)	0.000
PaO <sub>2</sub> /FiO <sub>2</sub>	172.0 (102.1–350.0)	104.8 (94.6–119.0)	371.7 (350.0–422.7)	0.001
>300, n/N (%)	3/10 (30.0%)	0/6 (0.0%)	4/4 (100.0%)	0.007
200–300, n/N (%)	2/10 (20.0%)	1/6 (16.7%)	0/4 (0.0%)	
100–200, n/N (%)	2/10 (20.0%)	2/6 (33.3%)	0/4 (0.0%)	
≤100, n/N (%)	3/10 (30.0%)	3/6 (50.0%)	0/4 (0.0%)	

Data are the median (IQR), n (%), or n/N (%), where N is the total number of patients with available data. P values comparing severe cases and moderate cases are from  $\chi^2$  test, Fisher's exact test, or unpaired 2-sided Student's t test. COVID-19, coronavirus disease 2019; FiO<sub>2</sub>, inspiratory oxygen fraction; IQR, interquartile range; PaO<sub>2</sub>, arterial oxygen tension; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SpO<sub>2</sub>, percutaneous oxygen saturation.

of breath, and acute respiratory distress syndrome. Additionally, a study of the first 41 laboratory-confirmed cases with COVID-19 showed that 63% of patients had lymphopenia, and cytokine storm could be associated with disease severity. However, information on immunological features between severe and moderate COVID-19 is scarce (8).

In this study, we performed a comprehensive evaluation of characteristics of 21 patients with COVID-19 admitted to Tongji Hospital, Wuhan. We aimed to compare the clinical and immunological features between severe cases and moderate cases. These findings may help us extend our understanding of the risk factors associated with disease severity in the SARS-CoV-2 infection.

## Results

*Patient demographics and baseline characteristics of severe and moderate COVID-19.* As of January 27, 2020, a total of 21 admitted

hospital patients with pneumonia were identified as laboratory-confirmed SARS-CoV-2 infection at Tongji Hospital. Of these patients, only 4, including a familial cluster of 3 confirmed cases, had direct exposure to the Huanan Seafood Market. According to the guidelines for diagnosis and management of COVID-19 (6th edition, in Chinese) issued by the National Health Commission of China (9), 11 (52.4%) patients with percutaneous oxygen saturation (SpO<sub>2</sub>) of 93% or lower or respiratory rates of 30/min or greater on room air who required high-flow nasal cannula or non-invasive mechanical ventilation using the bilevel positive airway pressure (BiPAP) mode to correct hypoxemia, were classified as having severe COVID-19, whereas 10 (47.6%) patients not reaching the criteria for severe COVID-19 were considered moderate. There were more male patients in both severe cases and moderate cases. The median age of the severe cases (61.0 years) was significantly higher than moderate cases (52.0 years) (Table 1). More

**Table 2. Laboratory findings and chest CT images of patients with COVID-19**

	Normal range	All patients (n = 21)	Severe cases (n = 11)	Moderate cases (n = 10)	P value
White blood cell count, ×10 <sup>9</sup> /L	3.5–9.5	5.7 (4.6–8.3)	8.3 (6.2–10.4)	4.5 (3.9–5.5)	0.003
<4, n (%)		3 (14.3%)	0 (0.0%)	3 (30.0%)	0.017
4–10, n (%)		15 (71.4%)	8 (72.7%)	7 (70.0%)	
≥10, n (%)		3 (14.3%)	3 (27.3%)	0 (0.0%)	
Neutrophil count, ×10 <sup>9</sup> /L	1.8–6.3	4.8 (2.8–6.9)	6.9 (4.9–9.1)	2.7 (2.1–3.7)	0.002
Lymphocyte count, ×10 <sup>9</sup> /L	1.1–3.2	0.9 (0.7–1.1)	0.7 (0.5–0.9)	1.1 (1.0–1.2)	0.049
<0.8, n (%)		9 (42.9%)	8 (72.7%)	1 (10.0%)	0.008
Hemoglobin, g/L	130–175	137.0 (127.0–147.0)	136.0 (125.5–144.5)	139.5 (132.8–146.0)	0.78
Platelet count, ×10 <sup>9</sup> /L	125–350	160.0 (137.0–189.0)	157.0 (134.0–184.5)	175.6 (148.3–194.0)	0.88
<100, n (%)		1 (4.8%)	0 (0.0%)	1 (10.0%)	0.48
Alanine aminotransferase, U/L	≤41	26.0 (16.0–42.0)	42.0 (32.5–50.0)	16.0 (13.3–21.8)	0.000
Aspartate aminotransferase, U/L	≤40	27.0 (21.0–47.0)	47.0 (28.0–74.5)	24.0 (21.5–26.5)	0.014
>40, n (%)		6 (28.6%)	5 (45.5%)	0 (0.0%)	0.035
Albumin, g/L	35.0–52.0	33.7 (29.6–37.4)	29.6 (28.6–33.0)	37.2 (35.8–38.8)	0.013
<32 g/L, n (%)		8 (38.1%)	7 (63.6%)	1 (10.0%)	0.024
Total bilirubin, mmol/L	≤26	8.8 (6.8–10.3)	8.8 (7.9–10.5)	7.8 (6.4–9.5)	0.24
Blood urea nitrogen, mmol/L	3.1–8.0	5.1 (4.1–6.4)	6.1 (5.2–9.1)	4.0 (3.4–4.8)	0.015
Creatinine, μmol/L	59–104	81.0 (67.0–85.0)	82.0 (67.5–91.5)	76.5 (63.3–81.0)	0.21
Creatine kinase, U/L	≤190	73.0 (63.0–287.0)	214.0 (90.0–329.0)	64.0 (57.5–83.5)	0.16
Lactate dehydrogenase, U/L	135–225	336.0 (221.0–537.0)	537.0 (433.5–707.5)	224.0 (200.3–251.8)	0.001
>300 U/L, n (%)		11 (52.4%)	10 (90.9%)	1 (10.0%)	0.000
Prothrombin time, seconds	11.5–14.5	13.7 (13.0–14.5)	14.3 (13.6–14.6)	13.4 (12.8–13.7)	0.15
Activated partial thromboplastin time, seconds	29.0–42.0	39.4 (33.6–44.5)	33.7 (32.1–38.4)	44.0 (42.6–47.6)	0.002
D-dimer, μg/mL	<0.5	0.5 (0.4–1.8)	2.6 (0.6–18.7)	0.3 (0.3–0.4)	0.029
Procalcitonin, ng/mL	0.02–0.05	0.11 (0.05–0.24)	0.18 (0.13–0.81)	0.05 (0.04–0.06)	0.059
<0.1, n/N (%)		7/18 (38.9%)	0/10 (0.0%)	7/8 (87.5%)	0.002
0.1–0.25, n/N (%)		6/18 (33.3%)	6/10 (60.0%)	0/8 (0.0%)	
0.25–0.5, n/N (%)		2/18 (11.1%)	1/10 (10.0%)	1/8 (12.5%)	
≥0.5, n/N (%)		3/18 (16.7%)	3/10 (30.0%)	0/8 (0.0%)	
High-sensitivity C-reactive protein, mg/L	<1	108.4 (28.0–139.5)	139.4 (86.9–165.1)	22.0 (14.7–119.4)	0.003
>60, n/N (%)		14/20 (70%)	11/11 (100.0)	3/9 (33.3%)	0.002
Ferritin, μg/L	30–400	1424.6 (337.4–1780.3)	1598.2 (1424.6–2036.0)	337.4 (286.2–1275.4)	0.049
>800, n/N (%)		12/19 (63.2%)	9/9 (100.0%)	3/10 (30.0%)	0.003
Bilateral involvement of chest CT scan on admission, n/N (%)		17/21 (81.0%)	10/11 (90.9%)	7/10 (70.0%)	0.31

Data are the median (IQR), n (%), or n/N (%), where N is the total number of patients with available data. P values comparing severe cases and moderate cases are from  $\chi^2$ , Fisher's exact test, or unpaired 2-sided Student's *t* test. COVID-19, coronavirus disease 2019; IQR, interquartile range; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

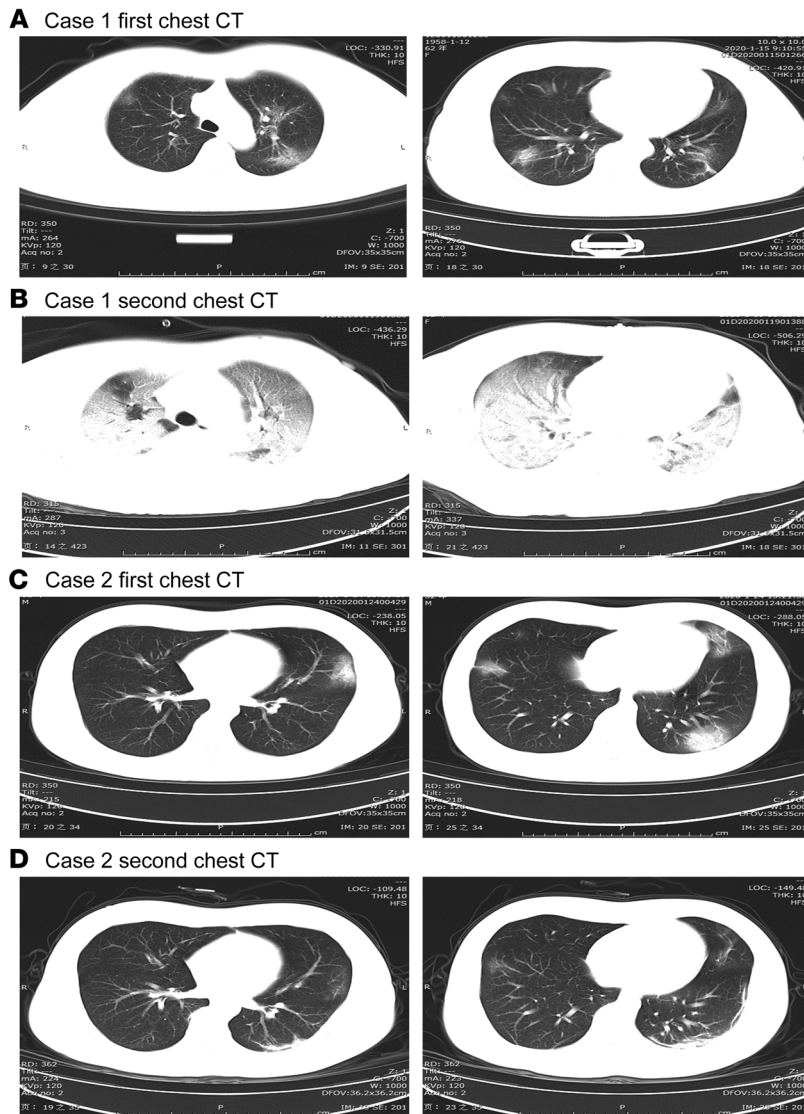
severe cases had comorbidity. The median time from onset of symptoms to first hospital admission was 8.0 days in severe cases and 7.0 days in moderate cases.

Four of 11 severe cases died an average of 20 days after the onset of the illness. Of these 4 deceased patients, all of them were male and aged 50 years and older, with 2 cases having hypertension. The median age of deceased cases was 64.0 years. Three of the deceased cases had an arterial oxygen tension/inspiratory oxygen fraction ratio (PaO<sub>2</sub>/FiO<sub>2</sub>) of 100 or less on admission.

Excluding 1 patient without a clear history due to a disorder of consciousness (coma) (classified as a severe case), the most common clinical manifestations at onset of illness included fever, cough, fatigue, and myalgia. Less common symptoms included sputum production, diarrhea, headache, and hemoptysis. Compared with moderate cases, chest tightness tended to be more com-

mon in severe cases. In addition, tachypnea and dyspnea were only developed in severe cases. All the severe cases developed dyspnea, and 9 of them with SpO<sub>2</sub> of 93% or lower showed no improved SpO<sub>2</sub> even with high-flow nasal cannula; these 9 cases were then ventilated using the BiPAP mode to treat hypoxemia. An arterial blood gas (ABG) test was performed in 10 patients on admission (6 severe and 4 moderate cases). Among them, the PaO<sub>2</sub>/FiO<sub>2</sub> ratio was significantly lower in severe cases (104.8) than moderate cases (371.7), with 3 out of 6 severe patients below 100.

*Laboratory findings and CT scans of severe and moderate COVID-19.* Compared with the normal range, the whole blood count on admission of 3 (30%) moderate cases showed mild leucopenia, while white blood cell (WBC) counts were normal or slightly increased above the upper limit of normal (ULN) in all the severe cases (Table 2). Both WBC and neutrophil counts were



**Figure 1. Computed tomography of the chest of patients with COVID-19.** Chest CT axial view lung window from a 62-year-old female with severe COVID-19 showing bilateral ground-glass opacity and subsegmental areas of consolidation on day 6 after symptom onset (A), and typical presentation of a white lung appearance with bilateral multiple lobular and subsegmental areas of consolidation on day 8 after symptom onset (B). Chest CT axial view lung window from a 32-year-old male with moderate COVID-19 showing bilateral ground-glass opacity on day 7 after symptom onset (C), and resolved bilateral ground-glass opacity on day 11 after symptom onset (D).

patients, 10 (90.9%) severe cases and 7 (70%) moderate cases had bilateral involvement on admission (Table 2). The typical findings of chest CT images of severe COVID-19 on admission showed bilateral ground-glass opacity and subsegmental areas of consolidation (Figure 1A), then progressed rapidly with mass shadows of high density in both lungs (Figure 1B). Representative chest CT images of moderate COVID-19 showed bilateral ground-glass opacity (Figure 1C). Subsequent chest CT images (4 days later) revealed that the bilateral ground-glass opacity had been resolved (Figure 1D).

*Immunological features of severe and moderate COVID-19.* We assessed plasma cytokine levels to examine the presence of cytokine storm in these patients. Evaluation of serum cytokines on admission revealed that levels of interleukin 2R (IL-2R), IL-6, IL-10, and tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) were markedly higher in severe cases than in moderate cases (Figure 2 and Supplemental Table 1; supplemental material available online with this article; <https://doi.org/10.1172/JCI137244DS1>). IL-1 $\beta$  concentrations were undetectable (<5 pg/mL) in nearly all the patients with either severe or moderate COVID-19.

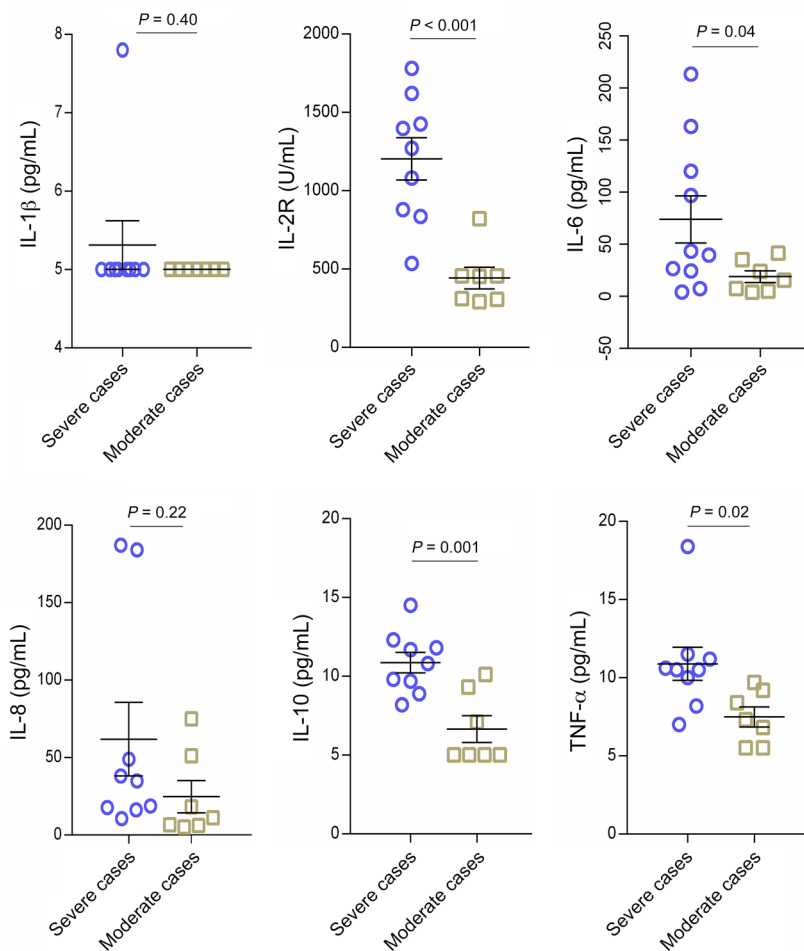
Overall, we found that macrophage-related proinflammatory cytokines, particularly IL-6, IL-10, and TNF- $\alpha$ , were significantly increased in the majority of severe cases. Of note, IL-6 levels were increased in both moderate and severe cases.

We next examined the proportions and effector functions of immune cells in peripheral blood (Figure 3 and Table 3). Preliminary analysis of circulating immune cell subsets, as shown in Table 3, demonstrated that absolute numbers of total T lymphocytes, CD4<sup>+</sup> T cells, and CD8<sup>+</sup> T cells were reduced below the lower limit of normal (LLN) in the vast majority of patients with either severe or moderate COVID-19, and they were reduced more profoundly in severe cases (294.0, 177.5, and 89.0  $\times 10^6/L$ , respectively) than in moderate cases (640.5, 381.5, and 254.0  $\times 10^6/L$ , respectively) (Figure 3, A and B). The proportion of B cells was significantly higher in severe cases (20.2%) than in moderate cases (10.8%). This could be partly due to the more significant decrease in T lymphocytes in severe cases. In addition, 6 (75.0%) of 8 severe cases showed a broad, significant decrease in all the lymphocyte subsets excluding B cells, with total T lymphocyte counts below 400  $\times 10^6/L$ , CD8<sup>+</sup> T cell counts below 150  $\times 10^6/L$ ,

significantly higher in severe cases than moderate cases, whereas lymphocyte counts were significantly lower in severe cases ( $0.7 \times 10^9/L$ ) than moderate cases ( $1.1 \times 10^9/L$ ). Lymphopenia (lymphocyte count  $<0.8 \times 10^9/L$ ) was developed in 8 (72.7%) severe cases and only 1 (10.0%) moderate case ( $P = 0.008$ ). Overall, severe cases had increased WBC counts ( $P = 0.003$ ) but lower lymphocyte counts ( $P = 0.049$ ).

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were significantly higher in severe cases than moderate cases. Albumin concentrations were significantly lower in severe cases than moderate cases, and hypoalbuminemia (albumin  $< 32$  g/L) was more frequent in severe cases (Table 2). Levels of lactate dehydrogenase (LDH), concentrations of serum high-sensitivity C-reactive protein (hsCRP), ferritin, and D-dimer levels were markedly higher in severe cases than moderate cases. In addition, serum levels of procalcitonin tended to be higher in severe cases than in moderate cases. These results suggest an increased level of systemic inflammation in severe cases.

Interstitial lung abnormalities were observed in chest computed tomography (CT) scans of all patients on admission. Of the 21



**Figure 2. Plasma cytokine levels in patients with COVID-19.** Series of comparisons of plasma cytokine levels between severe cases ( $n = 9$ ) and moderate cases ( $n = 7$ ). All data presented as the mean  $\pm$  SEM. Differences were tested using unpaired 2-sided Student's  $t$  test.

and natural killer (NK) cell counts below  $77 \times 10^6/L$ . Of these 6 patients, 3 (50%) eventually died.

The frequencies of regulatory T cells (Tregs) ( $CD4^+CD25^+CD127^{lo}$ ) and  $CD45RA^+$  Tregs were reduced (below the LLN) in nearly all the severe and moderate cases, and the  $CD45RA^+$  Treg proportion was markedly lower in severe cases (0.5%) than in moderate cases (1.1%). The reduced expression of interferon  $\gamma$  (IFN- $\gamma$ ) by  $CD4^+$  T,  $CD8^+$  T, and NK cells below the LLN was observed in some patients with severe (50%, 16.7%, and 16.7%, respectively) or moderate COVID-19 (14.3%, 0%, and 14.3%, respectively). The expression of IFN- $\gamma$  by  $CD4^+$  T cells tended to be lower in severe cases (14.1%) than moderate cases (22.8%) (Table 3 and Figure 2C). However, there was no significant difference in terms of mean fluorescence intensity of IFN- $\gamma$  production by  $CD4^+$  T,  $CD8^+$  T, or NK cells (data not shown). Overall, we found a significant reduction in  $CD4^+$  T cell count and a borderline reduction in IFN- $\gamma$  expression in severe cases.

**Complications and clinical outcomes of COVID-19.** With regard to complications as shown in Supplemental Table 2, common complications observed in severe cases included acute respiratory distress syndrome (100.0% of patients with available ABG data) and respiratory failure (83.3%). Less common complications among the

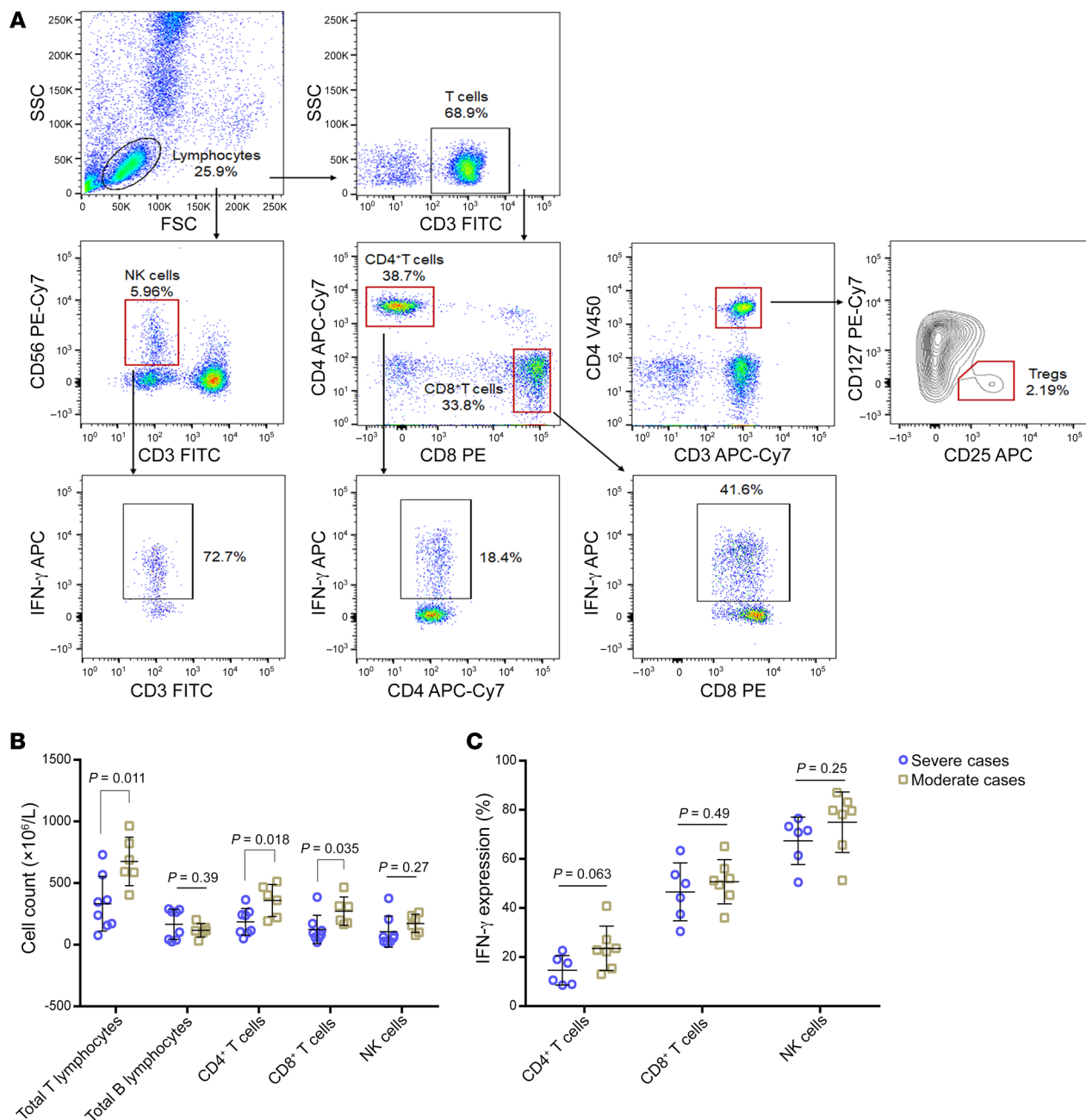
severe cases included secondary infection (27.3%), acute cardiac injury (9.1%), hypoxic encephalopathy (18.2%), acute kidney injury (18.2%), shock (9.1%), and acute liver injury (9.1%), most of which were not developed in any recovered cases.

All the severe and moderate cases were given empirical antimicrobial treatment (moxifloxacin and/or cephalosporin, etc.). Seven (63.6%) severe cases and all moderate cases received antiviral therapy (oseltamivir and/or ganciclovir). In addition, all severe and moderate cases were administered corticosteroid (methylprednisolone) during the course of hospitalization. Nine (81.8%) severe cases and no moderate cases required noninvasive mechanical ventilation. As of February 2, 2020, 4 (36.4%) of 11 severe cases and none (0.0%) of the moderate cases died, and the median number of days from illness onset to death was 20. One severe and 1 moderate case recovered. Patients were transferred to the designated hospital after being identified as having laboratory-confirmed SARS-CoV-2 infection.

### Discussion

This is the first preliminary study to our knowledge descriptively evaluating the immunological characteristics of patients with laboratory-confirmed SARS-CoV-2 infection. Both clinical and epidemiological features of patients with COVID-19 have recently been reported (5, 6, 8, 10). However, there is insufficient knowledge of pathophysiological parameters, particularly immunological indicators, to understand the mechanism involved in COVID-19. Consistent with previous reports (8), the present study showed that a male predominance in the incidence of COVID-19 has been noted, similarly to

that of SARS-CoV, indicating males are more susceptible to SARS-CoV-2 infection than females. Older males (>50 years old), particularly those with underlying comorbidities, may be more likely to develop severe COVID-19. The most common clinical manifestations at onset of illness included fever, cough, fatigue, and myalgia. Severe cases more frequently had dyspnea and developed acute respiratory distress syndrome. In terms of laboratory findings, leukocytosis ( $\geq 10 \times 10^9/L$ ) and lymphopenia ( $< 0.8 \times 10^9/L$ ) were more common in severe cases than in moderate cases. ALT, LDH, D-dimer, and inflammatory markers including hsCRP and ferritin were significantly higher in severe cases than in moderate cases. Serum concentrations of both proinflammatory cytokines and anti-inflammatory cytokines, including IL-2R, IL-6, TNF- $\alpha$ , and IL-10 increased in the majority of severe cases and were markedly higher than those in moderate cases, suggesting cytokine storms might be associated with disease severity. Similarly, SARS was also characterized by exuberant inflammatory responses and lung damage. A previous study using a mouse model of SARS demonstrated that rapid kinetics of SARS-CoV replication and delay in IFN-I signaling promoted inflammatory monocyte-macrophage accumulation, resulting in elevated lung cytokine/chemokine levels, vascular leakage, and suboptimal T cell responses (11). The underlying cel-



**Figure 3. Number of immune cell subsets and proportion of IFN- $\gamma$  expression in patients with COVID-19.** (A) Flow cytometric analysis of NK cells, CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells, and Tregs as well as production of IFN- $\gamma$  by CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells, and NK cells from a representative patient. (B) A series of comparisons of absolute number of total T and B lymphocytes, CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells, and NK cells between severe cases ( $n = 8$ ) and moderate cases ( $n = 6$ ). (C) A series of comparisons of production of IFN- $\gamma$  by CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells, and NK cells between severe cases ( $n = 6$ ) and moderate cases ( $n = 7$ ). All data presented as the mean  $\pm$  SEM. Differences were tested using unpaired 2-sided Student's  $t$  test.

lular origin and mechanism involved in cytokine accumulation in COVID-19 warrants further exploration.

Additionally, we noted that SARS-CoV-2 infection can cause a significant reduction in circulating lymphocytes and T cell subsets. Although the proportions of T cell subsets in peripheral blood remained within the normal range in most patients, decreased CD4<sup>+</sup> and CD8<sup>+</sup> T cell counts below the LLN were considerably frequent in both severe and moderate cases. More importantly, the number of CD4<sup>+</sup> and CD8<sup>+</sup> T cells was markedly lower in

severe cases than moderate cases. In contrast, both the proportion and number of B cells were not reduced in most patients, with 75.0% of severe cases showing an increased proportion of B cells. This could be partly due to the more significant decrease in T lymphocytes in these patients. It is notable that 6 out of 8 severe cases and none of the moderate cases with available immunological data exhibited a broad, significant decline in all the lymphocyte subsets, excluding B cells. Of these 6 patients, 3 eventually died. Moreover, the production of IFN- $\gamma$  by CD4<sup>+</sup> T cells but not CD8<sup>+</sup>

**Table 3. Immunological features of patients with COVID-19**

	All patients (n = 21)	Severe cases (n = 11)	Moderate cases (n = 10)	P value	Normal range
Total T lymphocytes, %	60.5 (54.4–70.3)	55.1 (52.2–60.5)	68.8 (64.7–75.2)	0.020	50–84
Total T lymphocyte count, ×10 <sup>6</sup> /L	486.5 (267.0–664.8)	294.0 (169.3–415.3)	640.5 (588.3–789.5)	0.011	955–2860
Decreased, n/N (%)	13/14 (92.9%)	8/8 (100.0%)	5/6 (83.3%)	0.43	
<400, n/N (%)	6/14 (42.9%)	6/8 (75.0%)	0/6 (0.0%)	0.010	
Total B lymphocytes (%)	16.9 (10.8–22.4)	20.2 (17.6–39.5)	10.8 (10.3–12.4)	0.025	5–18
Increased, n/N (%)	7/14 (50.0%)	6/8 (75.0%)	1/6 (16.7%)	0.10	
Total B lymphocyte count, ×10 <sup>6</sup> /L	115.5 (57.8–249.3)	184.0 (42.8–273.3)	115.5 (102.8–133.5)	0.35	90–560
Decreased, n/N (%)	4/14 (28.6%)	3/8 (37.5%)	1/6 (16.7%)	0.58	
CD4 <sup>+</sup> T cells, %	36.7 (32.0–40.0)	36.7 (30.7–37.3)	36.4 (32.0–40.6)	0.56	27–51
CD4 <sup>+</sup> T cell count, ×10 <sup>6</sup> /L	241.5 (135.0–363.8)	177.5 (104.0–249.8)	381.5 (255.0–451.0)	0.018	550–1440
Decreased, n/N (%)	14/14 (100.0%)	8/8 (100.0%)	6/6 (100.0%)	NA	
CD8 <sup>+</sup> T cells, %	22.2 (15.7–26.9)	17.4 (14.7–23.4)	25.2 (22.8–34.2)	0.093	15–44
CD8 <sup>+</sup> T cell count, ×10 <sup>6</sup> /L	169.5 (86.0–281.5)	89.0 (61.5–130.3)	254.0 (183.3–312.8)	0.035	320–1250
Decreased, n/N (%)	12/14 (85.7%)	7/8 (87.5%)	5/6 (83.3%)	1.00	
<150, n/N (%)	6/14 (42.9%)	6/8 (75.0%)	0/6 (0.0%)	0.010	
NK cells, %	14.8 (10.3–21.9)	14.7 (7.5–21.0)	15.1 (11.6–22.8)	0.62	7–40
NK cell count, ×10 <sup>6</sup> /L	89.0 (58.8–207.0)	60.5 (27.5–109.0)	180.5 (115.0–228.0)	0.27	150–1100
Decreased, n/N (%)	8/14 (57.1%)	6/8 (75.0%)	2/6 (33.3%)	0.28	
<77, n/N (%)	6/14 (42.9%)	6/8 (75.0%)	0/6 (0.0%)	0.010	
CD28 <sup>+</sup> CD4 <sup>+</sup> T cells/CD4 <sup>+</sup> T, %	98.3 (96.8–98.8)	97.5 (96.8–98.7)	98.6 (97.2–99.0)	1.00	84.11–100.00
CD28 <sup>+</sup> CD8 <sup>+</sup> T cells/CD8 <sup>+</sup> T, %	64.8 (44.6–75.9)	44.6 (37.5–73.1)	70.3 (63.3–76.9)	0.20	48.04–77.14
HLA-DR <sup>+</sup> CD8 <sup>+</sup> T cells/CD8 <sup>+</sup> T, %	42.3 (30.9–48.2)	46.2 (42.3–48.2)	28.6 (25.4–37.9)	0.19	20.73–60.23
CD45RA <sup>+</sup> CD4 <sup>+</sup> T cells/CD4 <sup>+</sup> T, %	32.8 (31.7–40.3)	32.8 (31.8–36.4)	36.0 (29.3–40.5)	0.54	29.41–55.41
CD45RO <sup>+</sup> CD4 <sup>+</sup> T cells/CD4 <sup>+</sup> T, %	67.2 (59.7–68.3)	67.2 (63.6–68.2)	64.0 (59.5–70.7)	0.54	44.44–68.94
Treg, %	4.1 (3.5–4.9)	4.7 (2.6–5.4)	3.9 (3.6–4.3)	0.92	5.36–6.30
CD45RA <sup>+</sup> Treg, %	0.8 (0.5–1.1)	0.5 (0.3–0.7)	1.1 (1.0–1.3)	0.020	2.07–4.55
CD45RO <sup>+</sup> Treg, %	3.3 (2.4–3.8)	3.8 (1.9–4.9)	2.9 (2.5–3.4)	0.59	1.44–2.76
IFN-γ-expressing CD4 <sup>+</sup> T cells, %	19.1 (13.0–22.8)	14.1 (9.4–18.8)	22.8 (18.8–25.4)	0.063	14.54–36.96
IFN-γ-expressing CD8 <sup>+</sup> T cells, %	50.1 (44.2–53.6)	47.2 (39.2–52.7)	51.2 (47.3–54.1)	0.49	34.93–87.95
IFN-γ-expressing NK cells, %	73.3 (65.7–79.7)	71.2 (63.8–72.9)	79.7 (71.9–81.5)	0.25	61.2–92.65

Data are the median (IQR) or n/N (%), where N is the total number of patients with available data. P values comparing severe cases and moderate cases are from  $\chi^2$ , Fisher’s exact test, or unpaired 2-sided Student’s t test. COVID-19, coronavirus disease 2019; IQR, interquartile range; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

T cells or NK cells tended to be lower in severe cases than moderate cases. These data suggest that SARS-CoV-2 infection induces lymphopenia, particularly in CD4<sup>+</sup> and CD8<sup>+</sup> T cells, as well as suppressed IFN- $\gamma$  production by CD4<sup>+</sup> T cells, which correlates with disease severity of COVID-19.

Although the total Treg proportion was comparable between severe and moderate cases, severe cases showed a significantly lower proportion of CD45RA<sup>+</sup> naive Tregs (nTregs) and a slightly higher proportion of their memory counterparts, CD45RO<sup>+</sup> memory Tregs (mTregs). nTregs might be activated in the periphery by antigen and subsequently converted to mTregs, and thus are thought to represent precursor cells of antigen-experienced mTregs and possess an equivalently strong suppressive capacity as compared with mTregs (12). It is reported that peripheral homeostatic mechanisms are crucial in the control of Treg diversity and concomitantly in the maintenance of immune tolerance in healthy individuals. Disturbances within these mechanisms may have detrimental consequences and could contribute to the development of certain diseases, particularly autoimmune dis-

eases (12). Whether the altered Treg proportions observed in the current study account for the severity of COVID-19, or correlate to the viremia, warrants further investigation.

CD4<sup>+</sup> T cells play a pivotal role in regulating immune responses, orchestrating the deletion and amplification of immune cells, especially CD8<sup>+</sup> T cells. CD4<sup>+</sup> T cells facilitate virus-specific antibody production via the T-dependent activation of B cells (13). However, CD8<sup>+</sup> T cells exert their effects mainly through 2 mechanisms, cytolytic activities against target cells or secretion of cytokines, including IFN- $\gamma$ , TNF- $\alpha$ , and IL-2, as well as many chemokines (14). The production of IFN- $\gamma$  is essential for the resistance against infection of various pathogens such as viruses, bacteria, and parasites (15). As a major source of IFN- $\gamma$ , the ability of T cells to respond to infection is part of the adaptive immune response that takes days to develop a prominent IFN- $\gamma$  response.

In this study, although decreased numbers of CD8<sup>+</sup> T cells were detected in severe cases, the proportion of CD8<sup>+</sup>HLA-DR<sup>+</sup> T cells was slightly greater than that in moderate cases, which is in agreement with a recent case report (16). Circulating CD8<sup>+</sup> T cells



were found to harbor high concentrations of cytotoxic granule components, including perforin and granulysin (16). Furthermore, a cytokine storm was exhibited in nearly all these populations; the only currently available histological examination of a severe case who died of SARS-CoV-2 demonstrated lung interstitial mononuclear inflammatory infiltrates, dominated by lymphocytes, and multinucleated syncytial cells with atypical enlarged pneumocytes in the intra-alveolar spaces (16). These findings suggested that overactivation of cytotoxic CD8<sup>+</sup> T cells, along with overproduction of proinflammatory cytokines, might account for, at least in part, the immunogenicity of COVID-19. Nevertheless, the cellular source (T cells, dendritic cells, or macrophages) of these cytokines remains to be determined.

The roles of T cell responses in the context of SARS-CoV and MERS-CoV infection have been previously studied. Patients who survived SARS-CoV and MERS-CoV infections usually had better immune responses than those who did not (17). The immune system plays an important role in both diseases, but it is differentially affected by the 2 viruses (18). A study in a mouse SARS-CoV model has shown that depletion of CD8<sup>+</sup> T cells at the time of infection does not affect viral replication or clearance. However, depletion of CD4<sup>+</sup> T cells leads to an enhanced immune-mediated interstitial pneumonitis and delayed clearance of SARS-CoV from the lungs, demonstrating the vital role of CD4<sup>+</sup> T cells but not CD8<sup>+</sup> T cells in primary SARS-CoV infection (19). A Chinese study in SARS-CoV-infected patients has demonstrated that the majority of infiltrative inflammatory cells in the pulmonary interstitium are CD8<sup>+</sup> T cells that play an important role in virus clearance as well as in immune-mediated injury (20). After comparing T cell-deficient mice and B cell-deficient mice, it was found that T cells are able to survive and kill virus-infected cells in the MERS-CoV-infected lung (21). These data highlight the importance of T lymphocytes, CD4<sup>+</sup> T cells in particular, but not B cells in controlling and fine-tuning the pathogenesis and outcomes of SARS-CoV and MERS-CoV infection. However, a cohort study investigating adaptive immune responses to SARS-CoV infection revealed that despite no significant correlation between the total T cell responses and disease progression, the disease severity correlates strongly with high-level CD4<sup>+</sup> T cell responses but not the CD8<sup>+</sup> memory T cell response (22). It is noteworthy that the immune responses evaluated in this study were in patients who recovered fully; thus, whether these responses contribute to recovery or disease progression remains unclear (22).

Chu et al. demonstrated that MERS-CoV, but not SARS-CoV, can efficiently infect T cells from the peripheral blood and from human lymphoid organs and induce apoptosis in T cells, which involves the activation of both the extrinsic and intrinsic apoptosis pathways (23). This may partly explain the lymphopenia observed in MERS-CoV-infected patients (23). SARS-CoV can also significantly decrease peripheral CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocyte subsets and this was related to the onset of illness (24). Several potential mechanisms may be involved, including the development of autoimmune antibodies or immune complexes triggered by viral infection and directly infecting and promoting the growth inhibition and apoptosis of hematopoietic stem and progenitor cells. The use of glucocorticoids may also account for the decrease in lymphocytes in some SARS patients (25). At present, little is

known about the mechanism underlying the lymphopenia caused by SARS-CoV-2 infection. In this study, we could not exclude the possibility that some of the lymphopenia may be worse due to the use of steroids during hospitalization. Further research is required to determine the effects of corticosteroids on lymphocytes in the context of COVID-19.

Our study has some limitations. First, we mainly evaluated the number of T cell subsets and NK cells as well as their IFN- $\gamma$  production; the function of these cells and the role of activated macrophages and lymphocytes infiltrating the pulmonary interstitium remain to be elucidated. Second, this study only included a small number of patients; thus, the results should be interpreted with caution, and statistical nonsignificance may not rule out differences between severe and moderate cases. Third, because data regarding the viremic profile of SARS-CoV-2 are not available, further studies are needed to investigate the correlation between the viral load kinetics and the dynamics of cellular immune responses. Clarification of these questions will allow further dissection of the complex SARS-CoV-2 pathogenesis, with potential implications for the development of therapeutics and vaccines.

In conclusion, the SARS-CoV-2 infection induced cytokine storm and lymphopenia, particularly a decrease in CD4<sup>+</sup> and CD8<sup>+</sup> T cell counts, as well as suppressed IFN- $\gamma$  production by CD4<sup>+</sup> T cells, which might be correlated with disease severity of COVID-19. Gaining a deeper understanding of the factors that affect lymphocytes, particularly T lymphocyte counts and their association with the disease severity in patients with SARS-CoV-2 infection, is of importance for clinical management of COVID-19.

## Methods

### Study participants

From late December 2019 to January 27, 2020, a total of 21 cases who initially presented with fever or respiratory symptoms, with pulmonary infiltrates on chest CT scans in the isolation ward of the Department of Infectious Disease, Tongji Hospital, were later confirmed to be infected with SARS-CoV-2 by the local health authority. Four cases had a history of exposure to the Huanan Seafood Market.

We retrospectively evaluated and analyzed the medical history, physical examination, and hematological, biochemical, radiological, microbiological, and immunological evaluation results obtained from these 21 patients with COVID-19. Epidemiological, clinical, laboratory, and radiological characteristics and treatment as well as outcome data were obtained from electronic medical records. The data collection forms were reviewed independently by 2 researchers.

### Clinical classifications and complication definitions

According to the guidelines for diagnosis and management of COVID-19 (6th edition, in Chinese) released by National Health Commission of China (9), the clinical classifications of COVID-19 are as follows:

**Mild cases:** The clinical symptoms are mild and no pneumonia manifestation can be found in imaging.

**Moderate cases:** Patients have symptoms like fever and respiratory tract symptoms, etc., and pneumonia manifestation can be seen in imaging.

**Severe cases:** Meeting any of the following — respiratory distress, respiratory rate  $\geq 30$  breaths/min; SpO<sub>2</sub>  $\leq 93\%$  at rest; and PaO<sub>2</sub>/FIO<sub>2</sub>  $\leq$

300. Patients with greater than 50% lesion progression within 24 to 48 hours in pulmonary imaging should be treated as severe cases.

Critically ill cases: Meeting any of the following — respiratory failure occurs and mechanical ventilation is required, shock, and complications from other organ failure that require monitoring and treatment in the ICU.

Acute respiratory distress syndrome and shock were defined according to the interim guidance of WHO for SARS-CoV-2 (26).

Hypoxemia was defined as a  $\text{PaO}_2/\text{FiO}_2$  ratio of less than 300.

Acute kidney injury was identified and classified based on the highest serum creatinine level or urine output criteria according to the Kidney Disease Improving Global Outcomes (KDIGO) classification.

Acute liver injury was defined as jaundice with a total bilirubin level of 3 mg/dL or higher and an acute increase in ALT of at least 5 times the upper limit of the normal range and/or an increase in alkaline phosphatase of at least twice the upper limit of the normal range.

Cardiac injury was diagnosed if serum levels of cardiac biomarkers (e.g., troponin I) were greater than the 99th percentile upper reference limit, or new abnormalities were shown in electrocardiography and echocardiography.

Secondary infection including bacterial and fungal was diagnosed if the patients had clinical symptoms or signs of nosocomial pneumonia or bacteremia, and was combined with a positive culture of a new pathogen from a respiratory tract specimen or from blood samples taken 48 hours or more after admission.

#### Laboratory measurements

**Real-time reverse transcription PCR assay for SARS-CoV-2.** Respiratory specimens were collected by the local CDC and then shipped to designated authoritative laboratories to detect SARS-CoV-2. The presence of SARS-CoV-2 in respiratory specimens was detected by real-time reverse transcription (RT-PCR) methods. Primers and probe targeting the CoV envelope gene were used and the sequences were as follows: forward primer, 5'-TCAGAATGCCAATCTCCCCAAC-3'; reverse primer 5'-AAAGGTCCACCCGATACATTTGA-3'; and the probe 5'-CY5-CTAGTTACTAGCCATCCTTACTGC-3'-BHQ1. Conditions for the amplifications were 50°C for 15 minutes, 95°C for 3 minutes, followed by 45 cycles of 95°C for 15 seconds and 60°C for 30 seconds.

**Clinical laboratory measurements.** Initial clinical laboratory investigation included a complete blood count, serum biochemical test (including liver and renal function, creatine kinase, LDH, and electrolytes), coagulation profile, as well as immunological test (including serum cytokines, peripheral immune cell subsets, and the expression of IFN- $\gamma$  by immune cells). Respiratory specimens, including nasal and pharyngeal swabs or sputum, were tested to exclude evidence of other viral infections, including influenza, respiratory syncytial virus, avian influenza, parainfluenza, and adenovirus. Routine bacterial and fungal examinations were also performed.

**Cytokine measurement.** To explore the influence of SARS-CoV-2 infection on the secretion of cytokines, IL-1 $\beta$ , IL-2R, IL-6, IL-8 (also known as CXCL8), IL-10, and TNF- $\alpha$  were assessed in serum samples drawn shortly after hospital admission by chemiluminescence immunoassay (CLIA) performed on a fully automated analyzer (Siemens Immulite 1000, DiaSorin Liaison, or Roche Diagnostics Cobas e602) for all patients according to the manufacturers' instructions. CLIA kits for IL-1 $\beta$  (LKL11), IL-2R (LKIP1), IL-8 (LK8P1), IL-10 (LKXP1), and TNF- $\alpha$  (LKNF1) were purchased from DiaSorin. An IL-6 kit (05109442 190) was purchased from Roche Diagnostics.

#### Evaluation of peripheral blood immunological indicators

The proportions and numbers of NK, CD4<sup>+</sup> T, CD8<sup>+</sup> T, Treg, and B cells, and the expression of cell surface markers as well as IFN- $\gamma$  expression by CD4<sup>+</sup> T, CD8<sup>+</sup> T, and NK cells were studied in these patients with laboratory-confirmed SARS-CoV-2 infection. Flow cytometry antibodies against human surface and intracellular molecules are commercially available. The following antibodies were used: anti-CD28 (CD28.2, PE, 555729), anti-CD8 (RPA-T8, PE-Cy7, 557746), anti-CD45 (2D1, PerCP, 347464), anti-HLA-DR (G46-6, APC, 560744), anti-CD3 (SK7, APC-Cy7, 557832), anti-CD4 (RPA-T4, V450, 560345), anti-CD45RA (HI100, FITC, 555488), anti-CD45RO (UCHL1, PE, 5618898), anti-CD127 (HIL-7R-M21, PE-Cy7, 560822), anti-CD45 (2D1, PerCP, 347464), anti-CD25 (M-A251, APC, 561399), anti-CD3 (SK7, APC-Cy7, 557832), anti-CD4 (RPA-T4, V450, 560345), anti-CD3 (UCHT1, FITC, 561806), anti-CD8 (RPA-T8, PE, 555367), anti-CD56 (B159, PE-Cy7, 557747), anti-IFN- $\gamma$  (4S.B3, APC, 551385), and anti-CD4 (RPA-T4, APC-Cy7, 557871). All reagents were purchased from Becton, Dickinson, and Company (BD). All samples were detected by a BD FACSCanto II flow cytometry system and analyzed with the BD FACSDiva software.

The steps of intracellular staining for IFN- $\gamma$  in immune cells were as follows: GolgiStop (BD Biosciences, 554724) was added to cell cultures for 4 hours and then the cells were resuspended in FACS buffer for flow cytometry antibody staining. Peripheral blood mononuclear cells were stained for surface antibody at 4°C for 30 minutes and washed with FACS buffer followed by fixation/permeabilization (BD Cytofix/Cytoperm, 554722) at 4°C for 20 minutes in the dark. Fixed/permeabilized cells were washed twice with Perm/Wash buffer (BD Biosciences, 554723) and then thoroughly resuspended in 50  $\mu$ L of Perm/Wash buffer containing a predetermined optimal concentration of a fluorochrome-conjugated anti-IFN- $\gamma$  antibody or appropriate negative control and incubated at 4°C for 30 minutes in the dark. Cells were washed twice with Perm/Wash buffer and resuspended in FACS buffer prior to flow cytometric analysis.

#### Statistics

Continuous variables were expressed as median (IQR) and compared with the unpaired 2-sided Student's *t* test; categorical variables were expressed as number (%) and compared by  $\chi^2$  test or Fisher's exact test between moderate and severe case groups. A 2-sided  $\alpha$  of less than 0.05 was considered statistically significant. Statistical analyses were done using SPSS (version 19.0, IBM).

#### Study approval

The study was performed in accordance with Good Clinical Practice and the Declaration of Helsinki principles for ethical research. The study protocol was approved by the Institutional Review Board of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (Wuhan, China). Written informed consent was waived due to the rapid emergence of this infectious disease.

#### Author contributions

QN and DW designed the study and had full access to all data in the study and take responsibility for the integrity of data and the accuracy of the data analysis. GC and DW contributed to patient recruitment, data collection, data analysis, data interpretation,

literature searches, and writing of the manuscript. WG and YC had roles in patient recruitment, data collection, and clinical management. DH, HW, TW, Xiaoyun Zhang, HC, HY, Xiaoping Zhang, MZ, SW, JS, TC, MH, SL, XL, and JZ had roles in patient management, experiments, data collection, data analysis, and data interpretation. All authors contributed to data acquisition, data analysis, or data interpretation, and reviewed and approved the final version of the manuscript. GC, DW, WG, and YC share first authorship and DH, HW, TW, and Xiaoyun Zhang are co-second authors; the order in which they are listed was determined by workload.

## Acknowledgments

We thank all the patients and their families involved in this study, as well as numerous doctors, nurses, and civilians working together to fight against the SARS-CoV-2.

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