Profile of IgG and IgM antibodies against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

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Abstract

We profiled the serological responses to Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) nucleocapsid (N) protein and spike (S) glycoprotein. The majority of the patients developed robust antibody responses between 17 and 23 days after illness onset. Delayed, but stronger antibody responses were observed in critical patients.

Keywords: SARS-CoV-2; COVID-19; Serologic Responses; IgG; IgM

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Introduction

A novel coronavirus (SARS-CoV-2) causing an outbreak of infectious pneumonia (COVID-19) emerged in December 2019 [1, 2]. Because there is currently no specific immunity in the population, humans of all ages and races are susceptible to SARS-CoV-2 infection. The World Health Organization (WHO) has declared SARS-CoV-2 a pandemic, and as of Apr 18, 2020, a total of 2,160,207 confirmed COVID-19 cases and 146,088 related deaths had been reported [3]. Diagnosis relies on viral RNA detection by RT-PCR using nasopharyngeal (NP) swabs. Considering the existence of asymptomatic transmission and false negative results of PCR caused by sampling mistakes or sometimes low viral shedding of the NP [4], improvement of COVID-19 diagnostic assays are still needed. Similar to SARS-CoV and MERS-CoV, the understanding of antibody responses specific to SARS-CoV-2 in patients will be helpful for diagnosis, seroepidemiologic surveys, and pathogenesis studies. In this study, we investigated the humoral immunity of hospitalized patients, analyzed the profile of IgG and IgM antibodies against the SARS-CoV-2 in 41 COVID-19 patients between three and 43 days of their illness.

Methods

Study design

Between January 11, 2020 and February 10, 2020, 394 COVID-19 patients were admitted to The Third People's Hospital of Shenzhen. SARS-CoV-2 was confirmed by two repeated positive results from our hospital and local Chinese Centers for Disease Control and Prevention using two different commercial RT-PCR kits approved by the National Medical Products Administration (NMPA), according to the manufacturer's protocol. Forty-one patients with preserved serial serum samples were included in this study. Patients were classified using the following criteria: 1) mild cases: clinical symptoms were mild without manifestation of pneumonia on imaging; 2) moderate cases: fever, respiratory symptoms, and with radiological findings of pneumonia; 3) severe cases: meeting any one of the following criteria: respiratory distress, hypoxia (SpO2≤93%), or abnormal blood gas analysis: (PaO2<60mmHg, PaCO2>50 mmHg); 4) critical cases: meeting any one of the following criteria: Respiratory failure requiring mechanical ventilation, shock, or other organ failure that

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requires ICU care. 41 patients were then divided into three groups: mild and moderate (15 patients), severe (16 patients), and critical (10 patients). A total of 347 serum specimens from these patients (5-31 samples from each patient) were collected between three and 43 days of disease onset for routine clinical testing. Control sera were collected from 10 patients with influenza and 28 patients completing routine check-ups between February 4, 2020 and February 10, 2020 at our hospital. The control sera were tested for the presence of IgG and IgM simultaneously with COVID-19 sera by the same method. The study was approved by the Institutional Review Board of The Third People's Hospital of Shenzhen (number 2020-0036).

Antibody detection

IgG and IgM antibodies against SARS-CoV-2 were measured using iFlash-SARS-CoV-2 IgG/IgM chemiluminescent immunoassay kit (C86095G/C86095M, YHLO BIOTECH, Shenzhen). According to the instructions, the sensitivity and specificity of the kits was 90% and 95% for IgG, 80% and 95% for IgM. As a screening assay for COVID-19 diagnosis, combined nucleocapsid (N) protein and spike (S) glycoprotein were used as coated antigens to increase the sensitivity. The levels of IgG and IgM antibodies were positively correlated with the Relative Luminescence Unit (RLU), and were calculated as AU/mL. Briefly, the serum samples of both healthy patients and confirmed COVID-19 patients were tested. According to the receiver operating curve (ROC curve), the corresponding concentration point of AUC (area under the ROC curve) greater than 0.9 was defined as the cut-off point, and the level of this point was defined as 10AU / mL.

Data analysis

Scatter plots were drawn to illustrate the cumultative proportion of patients with IgG and IgM antibodies, and the corresponding levels of IgG and IgM antibodies in 41 patients. LOWESS (locally weighted scatterplot smoothing) curves were fitted to display and compare the trends of antibody responses among groups. One-way ANOVA analysis was used to compare the levels of antibodies among groups. Paired *t*-test was used to compare the seroconversion time for IgM and IgG antibody in

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individual patients. “GraphPad Prism 8” software was used for the construction of the charts and the statistical analysis.

Results

All controls enrolled in the study tested negative (Table S4). Basic demographic information of the study participants are described in Table S1. The median age was 62.0 years (IQR 42.0-66.0), 34.1% were males, 22% had at least one comorbidity, 51.2% had been to Wuhan city, and 21.9% had been to other cities in Hubei province. 97.6% of patients (40/41) were positive with IgG and 87.8% of patients (36/41) were positive with IgM. Given the fact that most of the early cases went to the hospital late (around eight days after illness onset), whose first serum specimens were already positive with SARS-CoV-2 IgG or IgM, seroconversions of IgG and IgM antibodies against SARS-CoV-2 were only observed in 16 (39.0%) and 21 (51.2%) patients, respectively (Figure 1A and 1B). The median time of seroconversion for IgG was 11 days (8 - 16) and for and IgM, 14 days (8 - 28) after disease onset. On an individual basis, the seroconversion time of IgG antibody was earlier than that of IgM antibody (12.45±4.36 vs. 13.75±4.60 days, p=0.0019, Table S2, and Figure S1). The level of IgG antibody reached the highest concentration on day 30, while the highest concentration of IgM antibody appeared on day 18, but then began to decline.

The trends in antibody production was analyzed among the three groups with different disease severities during the first six weeks after disease onset, as illustrated in Figure 1C and 1D. For IgG, the fitting curve of those in the critical group rose rapidly above the cut-off value from day seven and peaked on day 20, while the fitting curves of the non-critical groups rose gently from day five. Although the IgG level of those in the mild and moderate group was still rising on day 28, the IgG response of the critical group was significantly stronger than that of non-critical groups within 4 weeks after illness onset (p=0.0001, Table S3). For IgM, the fitting curve of the critical group rose above the cut-off value on day 10, peaked on day 23, then began to decline. However, the IgM levels of non-critical groups rose above the cut-off value as early as day five, peaked on day 16, then dereased.

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Discussions

The results of this study demonstrate the overall profile and seroconversion patterns of IgM and IgG antibodies after SARS-CoV-2 infection using a total of 347 serum samples collected from 41 COVID-19 patients. The kinetics of anti-SARS-CoV-2 antibodies should be helpful in epidemiologic surveys, and especially in clinical diagnoses since the immunoassays can efficiently compensate the false negative limitations of nucleic acid testing.

In the majority of the patients, there were antibody responses to SARS-CoV-2 during the first three weeks of the disease. The seroconversion time of IgG antibody was earlier than that of IgM antibody (Table S2 and Figure S1). The profile of antibodies against SARS-CoV-2 was comparable with previous findings of SARS-CoV infections [5, 6]. Li *et al.*, reported that both IgG and IgM antibody levels increased to detectable levels from the second week of illness in 20 SARS-CoV patients [5]. Similarly, Woo *et al.*, also observed that the seroconversion time for IgG was three days earlier than that for IgM after the SARS-CoV infection [6]. The negative IgM results in five patients were possibly caused by the window phase of antibody production, as serum specimens were collected between day three and day 13, thus longer surveillance is needed.

On the other hand, Park *et al.*, reported that early antibody response was associated with reduced disease severity in MERS-CoV infections [7]. Xu *et al.*, revealed that imbalance of the immune system was a pathogenesis factor from the pathological finding of a COVID-19 case [8]. Here, compared to non-critical groups, we also observed delayed IgG and IgM antibody responses in the critical group (Figure 1C and 1D). Moreover, the slope of the IgG antibody response was steeper in the critical group (Table S3), which might be a hint of inflammatory storm. The intervention window might be the second week after illness onset for most patients.

Our study has several limitations. Firstly, Liu *et al.* found that acute lung injury in Chinese macaques caused by SARS-CoV could be mediated by higher anti-spike IgG [9], and we detected high levels of IgG antibody in critical patients. Since we used combined N and S proteins as capture antigen to increase sensitivity of this assay, further studies are needed to separate the effects of specific anti-N and anti-S antibodies. Secondly, we did not test the possible cross-reactivity of our in-house

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assay with common human coronaviruses (e.g., hCoV-OC43 or others), MERS-CoV, or SARS-CoV. No SARS-CoV or MERS-CoV infections had been reported by any of the patients in the study, and the infection rate of common hCoV infections has been estimated to be as low as 0.8% in a previous study [10]. Thus, even if the cross-reactivity exists, it would have limited impact on the validity of these findings.

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NOTES

Contributions JQ and LL were responsible for the study design, data interpretation, literature search, and writing of the manuscript. CW, XL, and GZ performed the serological testing. XL, ZJ, and QZ were responsible for the clinical management, patient recruitment, and data collection. CW, ZJ, XL, and QZ collected and analyzed the data.

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Potential conflicts of interest. The authors declare no conflict of interest.

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Figure Legends

FIG. 1 Longitudinal profile of IgG and IgM antibodies to SARS-CoV-2 nucleocapsid protein and spike glycoprotein in COVID-19 patients. (A) Cumulative proportion of patients who seroconverted and the concentration level of anti-SARS-CoV-2 IgG in the sera of 16 patients. (B) Cumulative proportion of patients who seroconverted and the concentration level of anti-SARS-CoV-2 IgM in the sera of 21 patients. (C) The level (AU/mL) of anti-SARS-CoV-2 IgG in mild and moderate, severe, and critical COVID-19 patients during hospitalization. (D) The level (AU/mL) of anti-SARS-CoV-2 IgM in mild and moderate, severe, and critical COVID-19 patients during hospitalization.

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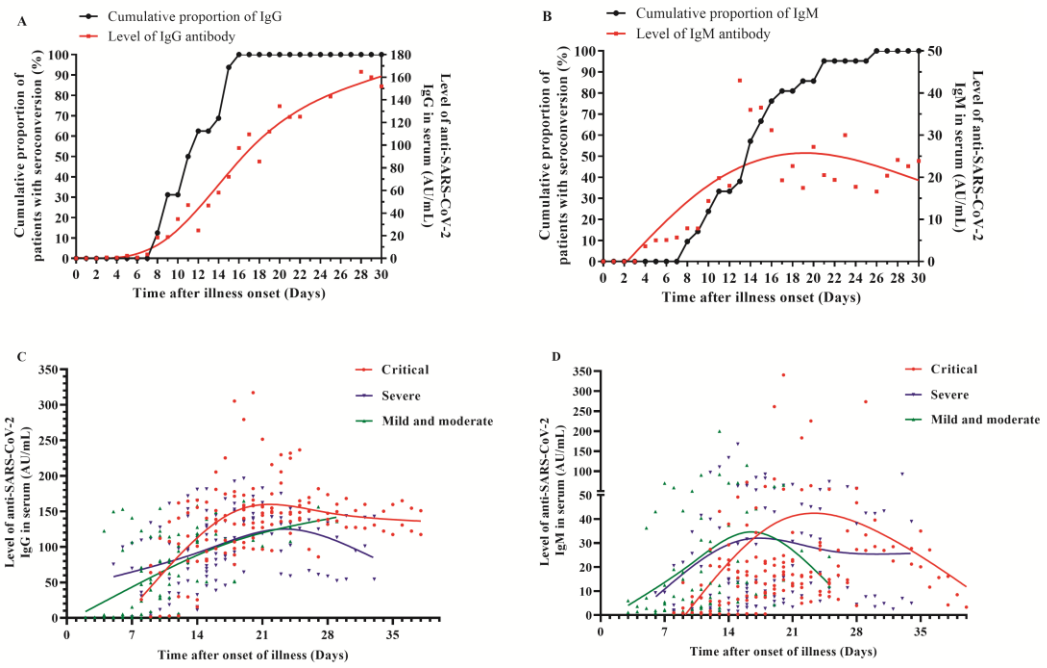
Figure 1



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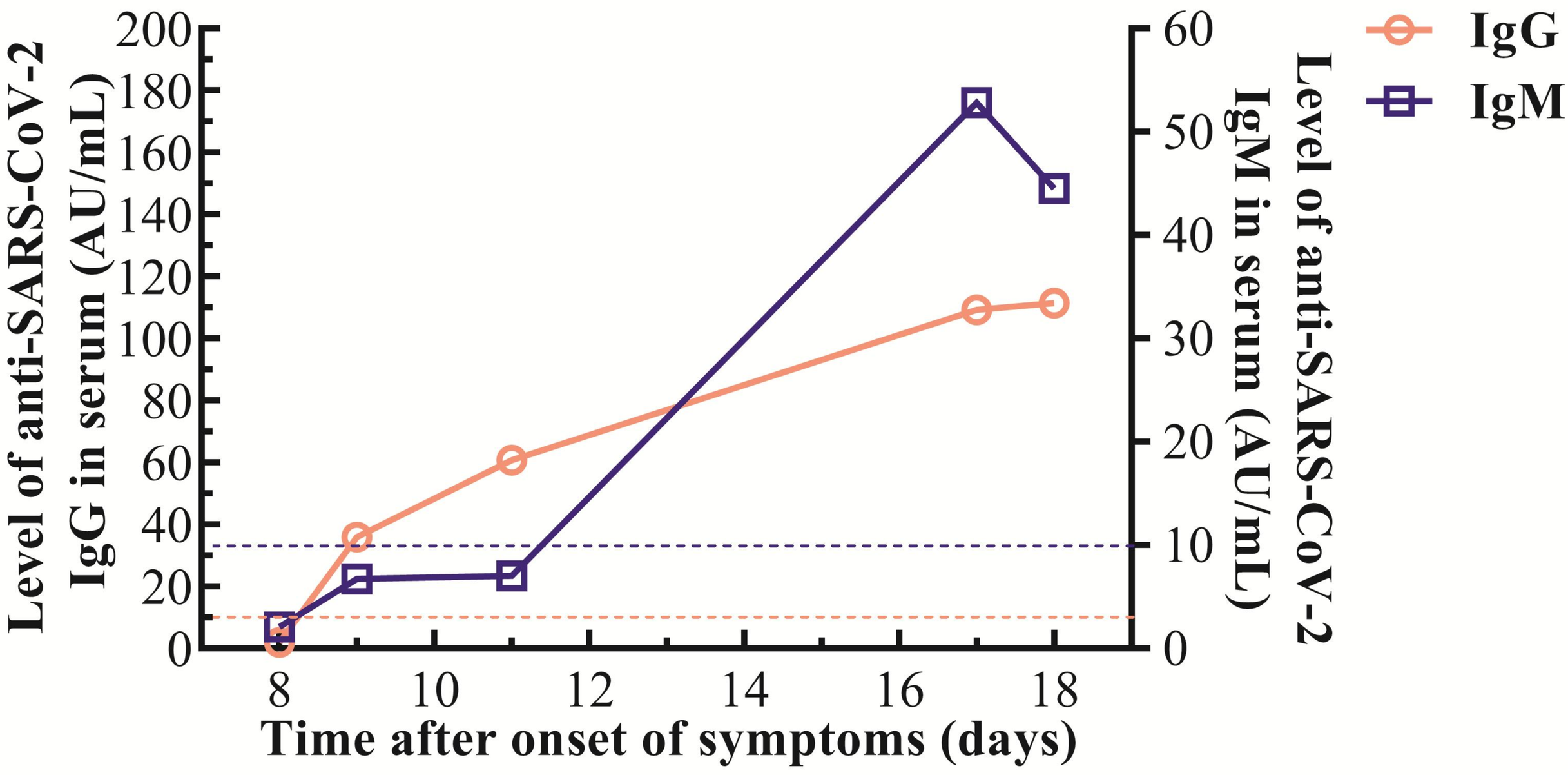
Case 8, Severe
Level of anti-SARS-CoV-2 IgG in serum (AU/mL)
Level of anti-SARS-CoV-2 IgM in serum (AU/mL)
Time after onset of symptoms (days)
IgG
IgM
0
20
40
60
80
100
120
140
160
180
200
0
10
20
30
40
50
60
8
10
12
14
16
18

Table S1. Demographics features of 41 COVID-19 patients on admission.

Characteristics	41 COVID-19 patients
Age, Median (IQR), years	62.0 (42.0-66.0)
Gender	
Male	27 (65.9%)
Female	14 (34.1%)
Epidemiological history	
Been to Wuhan city	21 (51.2)
Been to other cities in Hubei province	9 (21.9)
Contacted with people from Hubei province	4 (9.8)
Unclear	7 (17.1)
Comorbidity	9 (22%)
Signs and symptoms at admission	
Fever	35 (85.4%)
Cough	22 (53.7%)

Table S2. Comparison of the sero-conversion time for IgM and IgG antibodies in 41 COVID-19 patients

	SARS-CoV-2 IgG	SARS-CoV-2 IgM	*P* value
Sero-conversion time, days	12.45 (4.36)	13.75 (4.60)	0.0019

Note. Data were shown as Mean (SD).

Table S3. One-way ANOVA analysis comparing the levels of antibodies by severity among 41 COVID-19 patients

	Time after illness onset	Mild and moderate patients (n=15)	Severe patients (n=16)	*P* value [a]	Critical patients (n=10)	*P* value [b]	*P* value [c]	*P* value [d]
SARS-CoV-2 IgG (AU/mL)	3-43 Days	65.76 (54.46)	97.85 (50.72)	0.0001	134.60 (54.93)	0.0001	0.0001	0.0001
	2nd week	53.76 (46.15)	64.45 (53.06)	0.6263	79.65 (58.23)	0.1063	0.4924	0.1273
	3rd week	109.30 (37.10)	110.40 (46.47)	0.9964	145.50 (54.33)	0.0174	0.0009	0.0005
	4th week	125.60 (22.20)	122.90 (36.77)	0.9869	158.00 (36.68)	0.1503	0.0020	0.0017
SARS-CoV-2 IgM (AU/mL)	3-43 Days	21.38 (30.80)	41.56 (57.25)	0.0183	31.15 (58.75)	0.3621	0.2487	0.0238
	2nd week	21.49 (35.43)	39.11 (64.56)	0.1803	13.06 (12.29)	0.7161	0.0626	0.0598
	3rd week	29.16 (29.84)	49.66 (64.88)	0.4980	40.11 (78.80)	0.8171	0.7486	0.5021
	4th week	21.84 (12.11)	36.59 (31.65)	0.7354	31.07 (45.12)	0.8766	0.8664	0.7305

Note. Data were shown as Mean (SD). [a]: Mild and moderate vs. Severe; [b]: Mild and moderate vs. Critical; [c]: Severe vs. Critical; [d] Comparison among the three group.

Table S4. Demographic characteristics and serological test results of 38 negative controls.

Number	type	Gender	Age (years)	SARS-CoV-2 IgG (AU/mL)	SARS-CoV-2 IgM (AU/mL)
MY370	check-up	Female	35	6.59	1.49
MY389	check-up	Female	45	1.00	1.77
MY391	check-up	Male	45	0.74	4.28
MY392	check-up	Male	37	0.97	3.21
MY393	check-up	Female	30	1.58	0.70
MY394	check-up	Male	38	1.69	1.15
MY395	check-up	Female	22	1.45	1.26
MY396	check-up	Male	28	3.27	2.14
MY397	check-up	Female	33	4.40	6.66
MY398	check-up	Female	23	0.92	3.68
MY399	FluA	Male	31	2.18	0.36
MY400	FluA	Male	7	0.84	1.41
MY401	FluA	Male	5	0.70	1.68
MY402	FluA	Female	2	1.12	1.05
MY403	FluA	Female	4	0.95	1.52
MY404	FluA	Female	56	0.84	0.61
MY405	FluA	Male	7	1.11	1.77
MY406	FluA	Male	86	0.99	1.68
MY407	FluA	Male	6	0.79	0.88
MY408	FluA	Female	3	1.99	3.24
MY441	check-up	Male	40	0.39	0.59
MY442	check-up	Female	34	0.45	0.66
MY443	check-up	Female	56	0.41	0.90
MY445	check-up	Male	46	3.68	1.07
MY451	check-up	Female	24	0.97	1.15
MY453	check-up	Female	27	0.49	1.50
MY454	check-up	Male	46	4.10	0.58
MY460	check-up	Female	30	0.49	2.91
MY462	check-up	Male	44	0.33	0.44
MY463	check-up	Female	23	0.29	0.83
MY464	check-up	Male	27	0.37	0.80
MY465	check-up	Female	36	0.66	1.37
MY466	check-up	Female	47	0.99	1.51
MY467	check-up	Female	36	6.66	0.67
MY468	check-up	Male	46	0.36	0.64
MY471	check-up	Female	22	0.42	0.53
MY472	check-up	Female	34	0.72	0.89
MY474	check-up	Male	38	1.36	1.27