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Neutralizing Antibody Responses to Severe Acute Respiratory Syndrome Coronavirus 2 in Coronavirus Disease 2019 Inpatients and Convalescent Patients

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Background. Coronavirus disease 2019 (COVID-19) is a pandemic with no specific antiviral treatments or vaccines. There is an urgent need for exploring the neutralizing antibodies from patients with different clinical characteristics.

Methods. A total of 117 blood samples were collected from 70 COVID-19 inpatients and convalescent patients. Antibodies were determined with a modified cytopathogenic neutralization assay (NA) based on live severe acute respiratory syndrome coronavirus 2 and enzyme-linked immunosorbent assay (ELISA). The dynamics of neutralizing antibody levels at different time points with different clinical characteristics were analyzed.

Results. The seropositivity rate reached up to 100.0% within 20 days since onset, and remained 100.0% till days 41–53. The total geometric mean titer was 1:163.7 (95% confidence interval [CI], 128.5–208.6) by NA and 1:12 441.7 (95% CI, 9754.5–15 869.2) by ELISA. The antibody level by NA and ELISA peaked on days 31–40 since onset, and then decreased slightly. In multivariate generalized estimating equation analysis, patients aged 31–45, 46–60, and 61–84 years had a higher neutralizing antibody level than those aged 16–30 years ($\beta = 1.0470$, $P = .0125$; $\beta = 1.0613$, $P = .0307$; $\beta = 1.3713$, $P = .0020$). Patients with a worse clinical classification had a higher neutralizing antibody titer ($\beta = 0.4639$, $P = .0227$).

Conclusions. The neutralizing antibodies were detected even at the early stage of disease, and a significant response was shown in convalescent patients.

Keywords. SARS-CoV-2; COVID-19; neutralizing antibody; convalescent patient.

The family Coronaviridae is comprised of large, enveloped, single-stranded, positive-sense RNA viruses that can infect a wide range of animals and human [1]. Two coronavirus pandemics in human have emerged in the past 2 decades. Severe acute respiratory syndrome coronavirus (SARS-CoV) was first recognized in 2003, causing a global outbreak [2]. It was followed by another pandemic event in 2012 designated as Middle East respiratory syndrome coronavirus [3]. In December 2019, emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) originating in Wuhan, China, has rapidly spread worldwide, and the World Health Organization (WHO) declared coronavirus disease 2019 (COVID-19) a pandemic. As of 12 April 2020, cases of COVID-19 have been reported in 211 countries and territories worldwide, with a total of 1 696 588 confirmed cases and 105 952 deaths [4]. Moreover, the number of confirmed cases continues to grow at a rapid rate, including the United States [5]. To date, the outbreak in China has been effectively controlled by widespread testing, quarantine of cases, contact tracing, and social distancing [6]. As of 12 April 2020, a total of 82 160 of COVID-19 patients were confirmed in China, of which 1156 remained hospitalized for treatment [7]. Despite supportive care and conventional antiviral therapies, neither antiviral treatments nor vaccines that could specifically target against COVID-19 have been achieved [8].

Neutralizing antibodies play an important role in virus clearance and have been considered as a key immune product for protection or treatment against viral diseases. The results from some studies indicated that using convalescent plasma on patients with Ebola, SARS-CoV, and H5N1 avian influenza were effective [9]; moreover, the COVID-19 Joint Investigation Report by China–WHO described that serum collected from COVID-19 convalescent patients can fully neutralize the cellular infectivity of the isolated virus [10]. In addition, Shen et al [11] reported that in 5 critically ill patients with COVID-19, administration of convalescent plasma containing neutralizing antibody was followed by improvement in their clinical status. These findings raise the hypothesis that using convalescent plasma transfusion could also be beneficial in COVID-19

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patients. However, immunity duration and changes on immunity levels of patients in the convalescent period remains largely unknown. Given the knowledge gap of this field, we determined that an updated analysis of antibody levels of patients with COVID-19 at different time points and severity of illness might help develop rapid diagnostic reagents, vaccines, drugs, and other treatments, and would be of great significance for the long-term control and treatment of COVID-19.

The purpose of the current study was to analyze the dynamics of neutralizing antibody levels at different times since onset from COVID-19 patients with different disease severity and convalescent patients, and to provide information for the scientific community to understand, detect, and treat COVID-19.

MATERIALS AND METHODS

Study Design and Subjects

The COVID-19 case definition and clinical classification based on severity were defined according to the New Coronavirus Pneumonia Prevention and Control Protocol for COVID-19 (seventh edition) released by the National Health Commission of China. Seventy COVID-19 patients were enrolled from hospital A and hospital B, of whom 12 were inpatients and 58 were convalescent patients. To study the dynamics of neutralizing antibody response, blood samples of patients were collected successively. Among 70 patients, only 8 were followed up and tested again after discharge from hospital. The 8 convalescent patients were selected to study longitudinal changes in antibody titers, including 4 in the mild group and 4 in the moderate group. Two patients were tested twice, 2 patients were tested 3 times, and 4 patients were tested 4 times. Together with 39 patients with only 1 blood sample collection, a total of 117 blood samples were analyzed in the study. The protocol of the study was reviewed and approved by the Medical Ethical Committee of Beijing Youan Hospital, Capital Medical University (approval number LL-2020-041-K). Before enrollment, written informed consent was obtained from each enrolled patient.

Clinical Measurements

The demographic characteristics, clinical manifestations, and underlying conditions of patients were collected. In addition, history of residence in or traveling to Wuhan within recent weeks was obtained.

Immunogenicity Assessment

The indicators for immunogenicity assessment included seropositivity rate and the geometric mean titer (GMT). We conducted neutralization assay (NA) to evaluate antibody level according to the Reed-Muench method on day 5. The presence of neutralizing antibody was determined by a modified cytopathogenic assay. Serum samples were inactivated at 56°C for 30 minutes and serially diluted with cell culture medium in 2-fold steps. The diluted serums were mixed with a virus suspension of 100 median tissue culture infective dose in 96-well plates at a ratio of 1:1, followed by 2 hours' incubation at 36.5°C in a 5% carbon dioxide (CO_2) incubator; $1–2 \times 10^4$ Vero cells were then added to the serum-virus mixture, and the plates were incubated for 5 days at 36.5°C in a 5% CO_2 incubator. Cytopathic effect of each well was recorded under microscopes, and the neutralizing titer was calculated by the dilution number of 50% protective condition. A titer of $\geq$ 1:4 indicated seropositivity. For calculation of GMT, antibody titers of < 1:8, > 1:512, and > 1:1024 were assigned values of 1:4, 1:(512 + 512/2), and 1:(1024 + 1024/2), respectively.

To double check seropositivity rate and the GMT, enzyme-linked immunosorbent assay (ELISA) was additionally conducted. Ninety-six-well microplates were coated with 1 μg/mL purified SARS-CoV-2 virus solution at 2°C–8°C overnight, and blocked with 1% bovine serum albumin for 2–4 hours at 37°C. Diluted sera (1:100) were applied to each well for 1 hour at 37°C, followed by incubation with goat antihuman antibodies conjugated with horseradish peroxidase for 1 hour at 37°C after 3 washes with phosphate-buffered saline. The plate was developed using TMB, followed by 2M sulfuric acid addition to stop the reaction. To determine the final result, the ELISA plate was read at 450/630 nm by ELISA plate reader.

Statistical Analysis

Mean with standard deviation was used for continuous variables, and number with percentage was used for categorical variables. Median with minimum and maximum was used to describe days for antibody testing of first sample since onset. Kruskal-Wallis rank-sum nonparametric method was used to compare log-transformed neutralizing antibody values. The comparison of categorical data was performed using χ^2 test or Fisher exact test. The association between antibody levels and potential factors (ie, sex, age, clinical classification, and time since onset of symptoms) was estimated by generalized estimating equation (GEE) model with logit link function, which took into account the correlation between repeated measurements of each patient. Hypothesis testing was 2-sided with an α value of .05. Analyses were conducted using SAS version 9.4 software (SAS Institute, Cary, North Carolina).

RESULTS

Characteristics of the Patients

Of the 70 patients enrolled into this study, 58 were recovered and discharged from hospital, and 12 were inpatients. The average age of the patients was 45.1 years (range, 16.0–84.0 years). A total of 58.6% were female. Thirty eight (54.2%) patients were residents or ever traveled in Wuhan, Hubei. The numbers of patients with a history of cardiovascular disease, diabetes, and hypertension were 2 (2.8%), 5 (7.1%), and 9 (12.9%), respectively. One (1.4%) patient had asymptomatic infection, 22 (31.4%) had mild clinical manifestations, 43 (61.5%) were moderate, and

the remaining 4 (5.7%) were in severe condition. Circulating C-reactive protein levels for inpatients and convalescent patients were 7.5 and 17.2 mg/L, respectively. For the neutralizing antibody test of the first sample since onset in this study, the median time was 33.0 days (range, 10.0–53.0 days), and the time of convalescent patients (35.0 days) was longer than that of inpatients (13.5 days) (Table 1).

Changes on Antibody Levels With Days Since Onset

The seropositivity rate reached up to 100.0% for 117 blood samples at different stages of illness both by NA and ELISA.

The total GMT by NA was 1:163.7 (95% confidence interval [CI], 128.5–208.6), of which 52.1% (61/117) had a titer between 1:64 and 1:512. The total GMT by ELISA was 1:12 441.7 (95% CI, 9754.5–15 869.2), of which 47.9% (56/117) had a titer between 1:4000 and 1:40 000. The antibody levels both by NA and ELISA at different time since onset were significantly different ($P = .0012$ and $P = .0417$, respectively), peaked on day 31–40 since onset, and then decreased slightly (Table 2).

Univariate GEE analysis showed that the neutralizing antibody level during day 31–40 was significantly higher than other phases. However, multivariate GEE analysis showed that the antibody level during days 31–40 was only higher than days 10–20 ($\beta = -.6276$, $P = .0201$) (Table 3).

Blood samples at different time since onset also showed differences in the distribution of neutralizing antibody titers (Table 2 and Figure 1). The proportion with a titer < 1:64 decreased with days since onset ($P_{trend} = .0061$), and the lowest was found during days 41–53. During days 41–53 since onset, 65.5% of samples had a titer between 1:64 and 1:512, not significantly different from other phases ($P = .0990$). The proportion with a titer ≥ 1:512 increased with days since onset ($P_{trend} = .0227$), and peaked the highest during days 31–40.

Dynamics of Antibody Titers in Convalescent Patients Since Onset

Among the 8 convalescent patients, days of neutralizing antibody tests since onset ranged from 12.0 to 60.0. During days 12–25, antibody titers of 4 patients were on an increasing curve, and 4 patients were on a declining curve (Figure 2A and 2B, respectively). During days 26–60, antibody titers showed a marked increase in 4 patients Figure 2A but a decrease in 3 patients Figure 2B. One patient remained at a stable titer of 1:128, and the antibody titer of 1 patient decreased from 1:1536 on day 20 to 1:48 on day 43 (patients g and f, respectively, Figure 2B).

Changes on Antibody Levels With Demographic Characteristics and Clinical Classification

The neutralizing antibody titers were similar in the 2 sex groups (1:168.6 [95% CI, 101.2–280.9] in men vs 1:185.6 [95% CI, 129.1–266.6] in women). The effect of sex was not statistically significant in either univariate ($P = .9426$) and multivariate ($P = .8543$) GEE analysis. A significant neutralizing antibody response was observed in older patients, with a GMT of 1:220.1 (95% CI, 71.8–674.8) compared to patients aged 16–30 years (1:71.0 [95% CI, 27.7–181.8]), 31–45 years (1:205.6 [95% CI, 145.2–291.1]), and 46–60 years (1:192.9 [95% CI, 111.7–333.2])

Table 1. Demographics and Clinical Characteristics of Patients With Coronavirus Disease 2019

Characteristic	Total	Inpatients	Convalescent Patients
No. of patients	70	12	58
Male sex, No. (%)	29 (41.4)	4 (33.3)	27 (43.1)
History of residence or traveling in Wuhan, No. (%)	38 (54.2)	5 (41.7)	33 (56.9)
History of cardiovascular disease, No. (%)	2 (2.8)	0	2 (3.4)
History of diabetes, No. (%)	5 (7.1)	0	5 (8.6)
History of hypertension, No. (%)	9 (12.9)	0	9 (15.5)
Clinical classification, No. (%)			
Asymptomatic	1 (1.4)	0 (0.0)	1 (1.7)
Mild	22 (31.4)	3 (25.0)	19 (32.8)
Moderate	43 (61.5)	8 (66.7)	35 (60.3)
Severe	4 (5.7)	1 (8.3)	3 (5.2)
Age, y	45.1 ± 14.2	42.7 ± 11.6	45.6 ± 14.7
Body temperature at admission, °C	37.1 ± 0.7	37.0 ± 0.7	37.0 ± 0.7
Systolic blood pressure, mm Hg	126.1 ± 16.9	130.2 ± 8.4	125.2 ± 17.9
Diastolic blood pressure, mm Hg	78.4 ± 12.4	81.5 ± 10.0	77.7 ± 2.8
White blood cell count, × 10^9/L	4.3 ± 1.4	4.1 ± 1.4	4.4 ± 1.4
Neutrophils, × 10^9/L	2.6 ± 1.2	2.5 ± 1.2	2.6 ± 1.2
Lymphocytes, × 10^9/L	1.3 ± 0.6	1.3 ± 0.5	1.3 ± 0.6
Monocytes, × 10^9/L	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
Platelets, × 10^{12}/L	202.0 ± 70.8	169.2 ± 50.2	209.4 ± 72.4
Circulating C-reactive protein, mg/L	15.3 ± 20.1	7.5 ± 11.8	17.2 ± 22.1
Days for antibody testing of first sample since onset, median (min, max)	33.0 (10.0, 53.0)	13.5 (10.0, 22.0)	35.0 (12.0, 53.0)

Data are expressed as mean ± standard deviation unless otherwise indicated.

Table 2. Seropositivity Rates and Antibody Levels in 117 Blood Samples at Different Times Since Onset

Variable	Total	Days Since Onset				*P* Value
		10–20	21–30	31–40	41–53	
No. of samples	117	29	23	36	29	
Seropositivity by NA						
Proportion	117/117	29/29	23/23	36/36	29/29	
Percentage, % (95% CI)	100.0 (96.9–100.0)	100.0 (88.1–100.0)	100.0 (85.2–100.0)	100.0 (90.3–100.0)	100.0 (88.1–100.0)	
Seropositivity by ELISA						
Proportion	117/117	29/29	23/23	36/36	29/29	
Percentage, % (95% CI)	100.0 (96.9–100.0)	100.0 (88.1–100.0)	100.0 (85.2–100.0)	100.0 (90.3–100.0)	100.0 (88.1–100.0)	
GMT (1:) by NA, value (95% CI)	163.7 (128.5–208.6)	96.3 (55.5–167.3)	111.5 (61.2–203.4)	271.2 (175.8–418.5)	201.7 (144.1–282.2)	.0012[a]
GMT (1:) by ELISA, value (95% CI)	12 441.7 (9754.5–15 869.2)	7001.6 (3859.2–12 702.7)	10 284.2 (4878.0–21 682.0)	17 730.3 (12 186.9–25 795.3)	15 720.3 (11 570.5–21 358.6)	.0417[a]
Proportions with titer by NA, % (95% CI)						
< 1:64	18.0 (11.5–26.1)	31.0 (15.3–50.8)	26.1 (10.2–48.4)	11.1 (3.1–26.1)	6.9 (.9–22.8)	.0061[b]
1:64 to < 1:512	52.1 (42.7–61.5)	58.6 (38.9–76.5)	52.2 (30.6–73.2)	36.1 (20.8–53.8)	65.5 (45.7–82.1)	.0990[c]
≥ 1:512	29.9 (21.8–39.1)	10.4 (2.2–27.4)	21.7 (7.5–43.7)	52.8 (35.5–69.6)	27.6 (12.7–47.2)	.0227[b]
Proportions with titer by ELISA, % (95% CI)						
< 1:4000	28.2 (20.3–37.3)	51.7 (32.5–70.6)	39.1 (19.7–61.5)	16.7 (6.4–32.8)	10.3 (2.2–27.4)	< .0001[b]
1:4000 to < 1:40 000	47.9 (38.5–57.3)	31.0 (15.3–50.8)	34.8 (16.4–57.3)	47.2 (30.4–64.5)	75.9 (56.5–89.7)	.0005[b]
≥ 1:40 000	23.9 (16.5–32.7)	17.3 (5.9–35.8)	26.1 (10.2–48.4)	36.1 (20.8–53.8)	13.8 (3.9–31.7)	.1484[c]

Abbreviations: CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; GMT, geometric mean titer; NA, neutralization assay.

[a] *P* value for GMT was calculated using Kruskal-Wallis rank-sum nonparametric method.

[b] *P* value for proportions with titer < 1:64 and ≥ 1:512 was calculated using trend χ^2 test.

[c] *P* value for proportions with titer between 1:64 and 1:512 was calculated using χ^2 test.

(*P* = .0359). In multivariate GEE analysis, patients aged 31–45, 46–60, and 61–84 years were more likely to have a higher antibody level than those aged 16–30 years (β = 1.0470, *P* = .0125; β = 1.0613, *P* = .0307; β = 1.3713, *P* = .0020).

Compared to the patients with asymptomatic or mild manifestations (GMT, 1:141.9 [95% CI, 79.5–253.2]), the neutralizing antibody levels were similar to patients with moderate or severe condition (GMT, 1:199.5 [95% CI, 141.8–280.5]).

Table 3. Univariate and Multivariate Generalized Estimating Equation Analysis of Factors Associated With Antibody Levels

Characteristic	Univariate Analysis		Multivariate Analysis		
	P Value	β Coefficient	Standard Error	(95% CI)	*P* Value
Sex					
Female vs male	.9426	0.0423	.2304	(−.4092 to .4938)	.8543
Age, y					
31–45 vs 16–30	.0183	1.0470	.4190	(.2258–1.8683)	.0125
46–60 vs 16–30	.0228	1.0613	.4912	(.0985–2.0241)	.0307
61–84 vs 16–30	.0061	1.3713	.4446	(.5000–2.2426)	.0020
Clinical classification					
Moderate or severe vs mild	.0753	0.4639	.2036	(.0649–.8630)	.0227
Days since onset					
10–20 vs 31–40	.0284	−0.6276	.2700	(−1.1569 to −.0983)	.0201
21–30 vs 31–40	.0410	−0.5152	.2765	(−1.0571 to .0267)	.0624
41–53 vs 31–40	.0152	−0.2075	.2616	(−.7203 to .3053)	.4277

Abbreviation: CI, confidence interval.

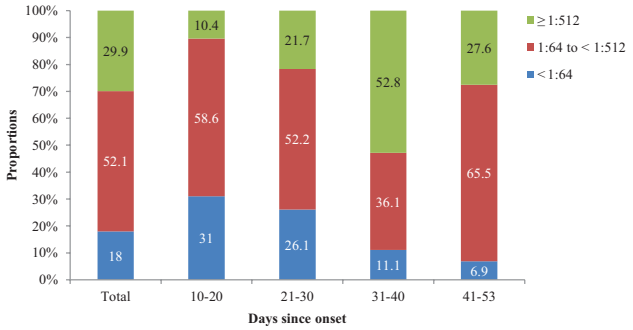

Figure 1. Distribution of neutralizing antibody titers in 70 patients at different times since onset.

However, after adjusting for other factors, patients with more severe symptoms tended to have a higher antibody titer (β = .4627, *P* = .0229).

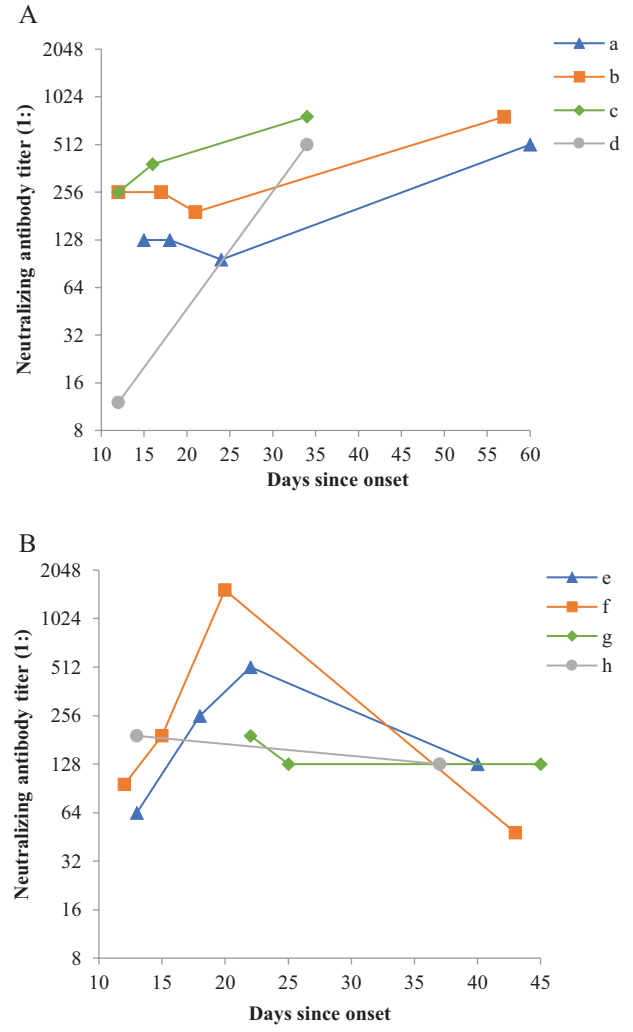

Figure 2. Dynamics of neutralizing antibody titers in 8 convalescent patients with coronavirus disease 2019 since onset; letters a–h represent the 8 convalescent patients, including 4 patients whose antibody titers showed an obvious increasing trend (*A*) and 4 patients whose antibody titers showed a decreasing trend (*B*).

Most convalescent patients showed higher GMT. Compared to inpatients (GMT, 1:76.1 [95% CI, 33.5–172.9]), the average antibody levels of convalescent patients were higher (GMT, 1:212.7 [95% CI, 157.5–287.3]; *P* = .0055). However, there were 3 convalescent patients with a GMT of 1:8, while the lowest GMT of inpatients was 1:6. Details are shown in Tables 3 and 4.

DISCUSSION

Due to COVID-19 widely spreading around the world, specific therapeutic agents and vaccines for COVID-19 are urgently needed. Neutralizing antibodies have been expected to be an effective measure to treat or prevent SARS-CoV-2 infection. Recent studies have demonstrated complete protection against SARS-CoV-2 with purified inactivated SARS-CoV-2 virus vaccine in macaques [12, 13]. Some studies used pseudovirus NA to evaluate the neutralizing antibody for SARS-CoV-2 [14–17]. To improve the performance of the test, we used NA based on live SARS-CoV-2. The results indicated a significant neutralizing antibody response in convalescent patients.

In the study, typical antibody responses to live viral infection were induced in all COVID-19 patients regardless of the stage of the disease. Moreover, the seropositivity rate can reach up to 100.0% on day 10. The GMT peaked between day 31 and day 40 after onset of symptoms. Even though the GMT had a slight decrease at days 41–53, the seropositivity rate remained 100.0%. The result was different from another study, which indicated that the titers of antibodies peaked between 10 and 15 days after disease onset [17]. After adjusting confounding factors, multivariate GEE analysis demonstrated that the antibody levels were comparable between days 31–40 and 41–53 since disease onset. However, the proportion with a titer of ≥1:512 decreased from

Table 4. Antibody Levels in 70 Patients by Sex, Age, Clinical Classification, and Recovery

Characteristic	GMT[a] (1:) (95% CI)	*P* Value[b]
Sex		
Male	168.6 (101.2–280.9)	.7243
Female	185.6 (129.1–266.6)	
Age, y		
16–30	71.0 (27.7–181.8)	.0359
31–45	205.6 (145.2–291.1)	
46–60	192.9 (111.7–333.2)	
61–84	220.1 (71.8–674.8)	
Clinical classification		
Asymptomatic or mild	141.9 (79.5–253.2)	.2435
Moderate or severe	199.5 (141.8–280.5)	
Recovery or not		
Inpatients	76.1 (33.5–172.9)	.0055
Convalescent patients	212.7 (157.5–287.3)	

Abbreviations: CI, confidence interval; GMT, geometric mean titer.

[a]The geometric mean of repeated measurements for each patient was used to represent the only testing result.

[b]*P* value for GMT was calculated using Kruskal-Wallis rank-sum nonparametric method.

52.8% on days 31–40 to 27.6% on days 41–53. How long antibody levels will last is a key concern for safe and effective antiviral treatments and vaccines in the future [18]. It is worthy of further study to analyze antibodies after COVID-19 patients have recovered for a longer time. For other coronaviruses, immunity after an infection is strong for several months [19]. Liu et al [20] found that the neutralizing activity infected by SARS pseudovirus declined from 96% at month 3 to 48% at month 36. Cao et al [21] showed that immunoglobulin G (IgG) and neutralizing antibodies were undetectable in 19.4% and 11.1% of serum samples at month 30 after onset, and in 25.8% and 16.1% of samples at month 36. It is uncertain whether the presence of antibodies against SARS-CoV-2, lower or even undetectable levels of specific neutralizing antibodies, could protect them from reinfection. Longitudinal observations in addition to stringent clinical and immunological characterization are needed to further assess the specificity and relative contribution to protection of neutralizing antibodies against SARS-CoV-2.

We found that the neutralizing antibody titers significantly increased along with age. Wu et al [17] also showed that elderly and middle-aged persons with COVID-19 had significantly higher plasma antibody titers and spike-binding antibodies than young patients. This indicated that elderly patients might have stronger immune response against SARS-CoV-2 than young patients. Whether high antibody levels protect these patients from progression into severe or critical conditions needs further study.

Our results indicated that convalescent patients had a higher antibody level than inpatients, which highlights the positive correlation between recovery and days since onset (Spearman correlation coefficient = 0.5426, $P < .0001$). However, 3 of the 58 patients recovered with a low level of GMT (1:8), not significantly higher than the lowest titer of inpatients (1:6), suggesting that besides neutralizing antibodies, other immune response, including T cells or cytokines, might contribute to convalescence [17].

Besides, we also found that neutralizing antibody levels in asymptomatic or mild cases were slightly lower than in moderate or severe cases, which matches with other previous studies [22, 23]. Zhang et al [23] concluded that severe cases were more frequently found in COVID-19 patients with high IgG levels, compared to those with low IgG levels. Previous data showed that severe SARS-CoV was also associated with more robust serological responses including early seroconversion and higher IgG levels [24, 25]. The GMT was 1:4 from the blood sample of the only patient with asymptomatic infection, lower than the lowest antibody level of symptomatic patients. However, the evidence that antibody levels of the asymptomatic infections are lower than those of symptomatic patients is not strong due to the small sample size in our study.

Several limitations of this study should be noted. First, the involved patients were selected by convenient sampling instead of random sampling. So the representativeness is relatively insufficient, and the samples could only represent the general situation to a certain extent. Second, among 70 patients, only 12 of them were followed up more than twice, and the average follow-up period was relatively short, about only 14.3 days (range, 3.0–36.0 days). Third, the subjects were mainly mild or moderate by illness severity, and only 1 asymptomatic patient and 4 severe patients were included. The neutralizing antibody response in asymptomatic infection and critical patients needs further exploration.

In conclusion, this study showed that all COVID-19 patients were seropositive to SARS-CoV-2 even at the early stage of illness, and a significant neutralizing antibody response was observed in convalescent patients. Neutralizing antibody levels depend on time after onset of symptoms, age, and the severity of disease.

Notes

Author contributions. Concept and design: Y. F. and Q. W. Acquisition, analysis, or interpretation of data: all authors. Drafting of the manuscript: X. W., X. G., and Q. X. Critical revision of the manuscript: Y. F. and Q. W. Laboratory testing: Y. H.,Y. P., and J. L. Data management and statistical analysis: Y. C. and Y. P.

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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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