Neutralizing Antibodies Responses to SARS-CoV-2 in COVID-19 Inpatients and Convalescent Patients

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Summary: Neutralizing Antibodies Responses to SARS-CoV-2 in Patients of COVID-19 Depends on Time since Onset and Severity of Disease.

Abstract

Background. COVID-19 is a pandemic with no specific antiviral treatments or vaccines. The urgent needs for exploring the neutralizing antibodies from patients with different clinical characteristics are emerging.

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 SARS-CoV-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 day 41-53. The total GMT was 1:163.7 (95% CI, 128.5 to 208.6) by NA and 1:12441.7 (95% CI, 9754.5 to 15869.2) by ELISA. The antibody level by NA and ELISA peaked on day 31-40 since onset, and then decreased slightly. In multivariate GEE analysis, patients at age of 31-45, 46-60, and 61-84 had a higher neutralizing antibody level than those at age of 16-30 (β=1.0470, P=0.0125; β=1.0613, P=0.0307; β=1.3713, P=0.0020). Patients with a worse clinical classification had a higher neutralizing antibody titer (β=0.4639, P=0.0227).

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

Keywords. SARS-CoV-2; COVID-19; Neutralizing antibody; Convalescent patient

Introduction

The family Coronaviridae is comprised of large, enveloped, single-stranded, and 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 two 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 (MERS-CoV) [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 April 12, 2020, the 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 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 April 12, 2020, a total of 82,160 of COVID-19 patients were confirmed in China, of which 1,156 remained hospitalized for treatment [7]. Despite supportive care and conventional anti-virus 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 researches indicated that using convalescent plasma on Ebola, SARS-CoV and H5N1 avian influenza patients were proved to be effective [9], moreover, COVID-19 Joint Investigation Report

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

The purpose of this current study was to analyze the dynamics of neutralizing antibody levels at different time since onset from different severity COVID-19 inpatients 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

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 (7th edition) released by the National Health Commission of China. Seventy COVID-19 patients were enrolled from A hospital and B hospital, 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 for another time after discharging from hospital. The 8 convalescent patients were selected to study longitudinal changes of antibody titers, including 4 in mild group and 4 in moderate group. Two patients were tested for twice, 2 patients were tested for three time, and 4 patients for four times. Together with 39 patients with only one 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, the 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 neutralizing assay (NA) to evaluate antibody level according to 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 two-fold steps. The diluted serums were mixed with a virus suspension of 100 TCID50 (50 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% CO_2 incubator. 1-2×10^4 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 (CPE) of each well was recorded under microscopes, and the neutralizing titer was calculated by the dilution number of 50% protective condition. A titer of 1:4 or higher 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 geometric mean titer (GMT), enzyme linked immunosorbent assay (ELISA) was additionally conducted. 96-well micro plates were coated with 1 μg/ml purified SARS-CoV-2 virus solution at 2-8℃ overnight, and blocked with 1% BSA for 2～4h at 37 ℃. Diluted sera(1:100) were applied to each well for 1h at 37℃ followed by incubation with goat anti-human antibodies conjugated with HRP for 1h at 37℃ after 3 times PBS wash. The plate was developed using TMB, followed by 2M H2SO4 addition to stop the reaction. To determine the final result, ELISA plate was read at 450/630 nm by ELISA plate reader.

Statistical Analysis

Mean with standard deviation was used for continuous variables description, and number with percentage was used for categorical variables description. Median with minimum and maximum was used to describe days for antibody testing of 1st 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 Chi-square test or Fisher's exact test. The association between antibody levels and potential factors, i.e., gender, age, clinical classification,

and time since onset of symptoms, were estimated by Generalized Estimating Equations (GEE) model with logit link function, which took into account the correlation between repeated measurements of each patient. Hypothesis testing was two-sided with an alpha value of 0.05. Analyses were conducted by SAS 9.4 (SAS Institute, Cary, NC, USA).

Role of the Funding Source

The funders had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Characteristics of the Patients

Of the 70 patients enrolled into this study, 58 were recovered and discharged from hospital, 12 were inpatients. The average age of the patients was 45.1 years (range 16.0-84.0). A total of 58.6% were female. Thirty eight (54.2%) patients were residents or ever travelled in Wuhan, Hubei. The number of patients with a history of cardiovascular disease, diabetes, and hypertension was 2 (2.8%), 5 (7.1%) and 9 (12.9%), respectively. One (1.4%) patient was 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 for inpatients and convalescent patients were 7.5 and 17.2 mg/L, respectively. For the neutralizing antibody test of 1st sample since onset in this study, the median time was 33.0 days (range 10.0-53.0), and the time of convalescent patients (35.0 days) were longer than 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% CI, 128.5 to 208.6), of which 52.1% (61/117) had a titer between 1:64 and 1:512. The total GMT by ELISA was 1: 12441.7 (95% CI, 9754.5 to 15869.2), of which 47.9% (56/117) had a titer between 1:4000 and 1:40000. The antibody levels both by NA and ELISA at different time since onset were significantly different (P=0.0012, P=0.0417), 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 day 31-40 was only higher than day 10-20 (β= -0.6276, P=0.0201) (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 less than 1:64 decreased with days since onset (P_{trend}=0.0061), and the lowest was found during day 41-53. During day 41-53 since onset, there were 65.5% of samples with a titer between 1:64 and 1:512, not significantly different from other phases (P=0.0990). The proportion with a titer of 1:512 or above increased with days since onset (P_{trend}=0.0227), and peaked the highest during day 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 day 12-25, antibody titers of 4 patients (c, d, e, and f) were on an increasing curve, however, 4 patients (a, b, g, and h) were on a declining curve (Figure 2). Then during day 26-60, antibody titers showed a marked increase in 4 patients (a, b, c, and d) but a decrease in 3 patients (e, f, and h). One patient (g) remained a stable titer of 1:128. It should be noted that the antibody titer of 1 patient (f) decreased from 1:1536 on day 20 to 1:48 on day 43.

Changes on Antibody Levels with demographic characteristics and Clinical Classification

The neutralizing antibody titers were similar in the two gender groups, of which 1:168.6 (95% CI, 101.2 to 280.9) in male and 1:185.6 (95% CI, 129.1 to 266.6) in female. The effect of gender was not statistically significant in both univariate (P=0.9426) and multivariate (P=0.8543) GEE analysis. A significant neutralizing antibody response was observed in older patients with a geometric mean titer of 1:220.1 (95% CI, 71.8 to 674.8) compared to patients at age of 16-30 (1:71.0, 95% CI, 27.7 to 181.8), 31-45 (1:205.6, 95% CI, 145.2 to 291.1) and 46-60 (1:192.9, 95% CI, 111.7 to 333.2) (P=0.0359). In multivariate GEE analysis, patients at age of 31-45, 46-60 and 61-84 were more likely to have a higher antibody level than those at age of 16-30 (β=1.0470, P=0.0125; β=1.0613, P=0.0307; β=1.3713, P=0.0020).

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

with more severe symptoms tended to have a higher antibody titer (β=0.4627, P=0.0229).

Most convalescent patients showed higher GMT. Compared to the inpatients (GMT 1:76.1, 95% CI, 33.5 to 172.9), the average antibody levels of convalescent patients were higher (GMT 1:212.7, 95% CI, 157.5 to 287.3) (P=0.0055). However, there were 3 convalescent patients with a GMT of 1:8, while the lowest GMT of inpatients was 1:6. Details were listed in Table 3 and 4.

Discussion

Due to the COVID-19 widely spreading around the world, the specific therapeutic agents or vaccines for COVID-19 are urgently needed. Neutralizing antibodies have been expected as an effective measure to treat or prevent SARS-CoV-2 infection. Recent researches demonstrated a complete protection against SARS-CoV-2 with purified inactivated SARS-CoV-2 virus vaccine in macaques [12,13]. Some studies used pseudovirus (PsV) neutralization assay to evaluate the neutralizing antibody for SARS-CoV-2 [14-17]. To improve the performance of the test, we used neutralization assay 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 the stage of the disease. Moreover, the seropositivity rate can reach up to 100.0% on day 10. The GMT peaked between day 31-40 after onset of symptoms. Even though the GMT had a slight decrease at day 41-53, the seropositivity rate remained 100.0%. The result was

different from another study which indicated the titers of antibodies peaked between 10 to 15 days after disease onset [17]. After adjusting confounding factors, multivariate GEE analysis demonstrated that the antibody levels were comparable between day 31-40 and day 41-53 since disease onset. However, the proportion with a titer of 1:512 or above decreased from 52.8% on day 31-40 to 27.6% on day 41-53. How long will antibody levels 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 recovered for a longer time. For other coronaviruses, immunity after an infection was strong for several months [19]. Liu[20] found that the neutralizing activity infected by SARS pseudovirus declined from 96% at month 3 to 48% at month 36. Cao [21] showed that 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% at month 36. It's uncertain whether the presence of antibodies against SARS-CoV-2, lower or even undetectable levels of specific neutralizing antibodies could protect them from re-infection. 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 [17] also showed that elderly and middle-age COVID-19 patients 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 studies.

Our results indicated that convalescent patients had a higher antibody level than inpatients, which highlight the positive correlation between recovery and days since onset (Spearman correlation coefficient=0.5426, P<0.0001). 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 cell or cytokines might contribute to the convalescence[17].

Besides, we also found that neutralizing antibody levels in asymptomatic or mild patients were slightly lower than moderate or severe patients, which matches with other previous studies [22,23]. Zhang [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 blood sample of the only one asymptomatic infection, lower than the lowest antibody level of symptomatic patients. However, the evidence that the antibody level of the asymptomatic infections are lower than that 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). 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 explored in future.

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 depends on time after onset of symptoms, age and the severity of disease.

Notes

Contributions

Concept and design: Yingmei Feng and Quanyi Wang.

Acquisition, analysis, or interpretation of data: All Authors.

Drafting of the manuscript: Xiaoli Wang, Xianghua Guo, and Qianqian Xin.

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Declaration of Interests

Authors certify no potential conflicts of interest.

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Table 1 Demographics and clinical characteristics of the COVID-19 patients.

Characteristic	Total	Inpatients	Convalescent patients
Number of patients	70	12	58
Male, n (%)	29 (41.4%)	4 (33.3%)	27 (43.1%)
History of residence or traveling in Wuhan, n(%)	38 (54.2%)	5 (41.7%)	33 (56.9%)
History of cardiovascular disease, n(%)	2 (2.8%)	0	2 (3.4%)
History of diabetes, n(%)	5 (7.1%)	0	5 (8.6%)
History of hypertension, n(%)	9 (12.9%)	0	9 (15.5%)
Clinical classification, n(%)			
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(years)	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	37.1±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 cells (10^9/L)	4.3±1.4	4.1±1.4	4.4±1.4
Neutrophil (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 1st 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±SD if not specified.

Table 2 Seropositivity rates and antibody levels in 117 blood samples at different time since onset.

Variable	Total	Days since onset				*P*
		10-20	21-30	31-40	41-53	
Number 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)	0.0012[a]
GMT(1:) by ELISA , value(95% CI)	12441.7 (9754.5-15869.2)	7001.6 (3859.2-12702.7)	10284.2 (4878.0-21682.0)	17730.3 (12186.9-25795.3)	15720.3 (11570.5-21358.6)	0.0417[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 (0.9-22.8)	0.0061[b]
1:64≤and <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)	0.0990[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)	0.0227[b]
Proportions with titer by ELISA, %(95% CI)						
<1:4000	28.2	51.7	39.1	16.7	10.3	<0.0001[b]

	(20.3-37.3)	(32.5-70.6)	(19.7-61.5)	(6.4-32.8)	(2.2-27.4)	
1:4000≤and <1:40000	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)	0.0005[b]
≥1:40000	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)	0.1484[c]

Note: NA: neutralization assay; ELISA: enzyme-linked immunosorbent assay

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

[b] *P* value for proportions with titer less than 1:64 and 1:512 or above was calculated using trend Chi-square test.

[c] *P* value for proportions with titer between 1:64 and 1:512 was calculated using Chi-square test.

Table 3 Univariate and multivariate GEE analysis of factors associated with antibody levels.

Characteristic		Univariate	Multivariate			
		P	Coefficient	Standard error	95% CI	*P*
Gender	Female *vs.* male	0.9426	0.0423	0.2304	-0.4092-0.4938	0.8543
Age(years)						
	31-45 *vs.* 16-30	0.0183	1.0470	0.4190	0.2258-1.8683	0.0125
	46-60 *vs.* 16-30	0.0228	1.0613	0.4912	0.0985-2.0241	0.0307
	61-84 *vs.* 16-30	0.0061	1.3713	0.4446	0.5000-2.2426	0.0020
Clinical classification	Moderate or severe *vs.* mild	0.0753	0.4639	0.2036	0.0649-0.8630	0.0227
Days since onset						
	10-20 *vs.* 31-40	0.0284	-0.6276	0.2700	-1.1569- -0.0983	0.0201
	21-30 *vs.* 31-40	0.0410	-0.5152	0.2765	-1.0571-0.0267	0.0624
	41-53 *vs.* 31-40	0.0152	-0.2075	0.2616	-0.7203-0.3053	0.4277

Table 4 Antibody levels in 70 patients by gender, age, clinical classification, and recovery.

Characteristic		GMT* (1:), value(95% CI)	*P* #
Gender			
	Male	168.6(101.2-280.9)	0.7243
	Female	185.6(129.1-266.6)	
Age(years)			
	16-30	71.0(27.7-181.8)	0.0359
	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)	0.2435
	Moderate or severe	199.5(141.8-280.5)	
Recovery or not			
	Inpatients	76.1(33.5-172.9)	0.0055
	Convalescent patients	212.7(157.5-287.3)	

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

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

Figure Legends

Figure 1

Distribution of neutralizing antibody titers in 70 patients at different time since onset.

Figure 2

Dynamics of neutralizing antibody titers in 8 convalescent COVID-19 patients since onset.

In the figure, a, b, c, d, e, f, g and h represented the 8 convalescent patients. In Figure 2-A, four patients whose antibody titers showed the obvious increasing trend were included. Figure 2-B included the other four patients whose antibody titers showed the decreasing trend.

Figure 1

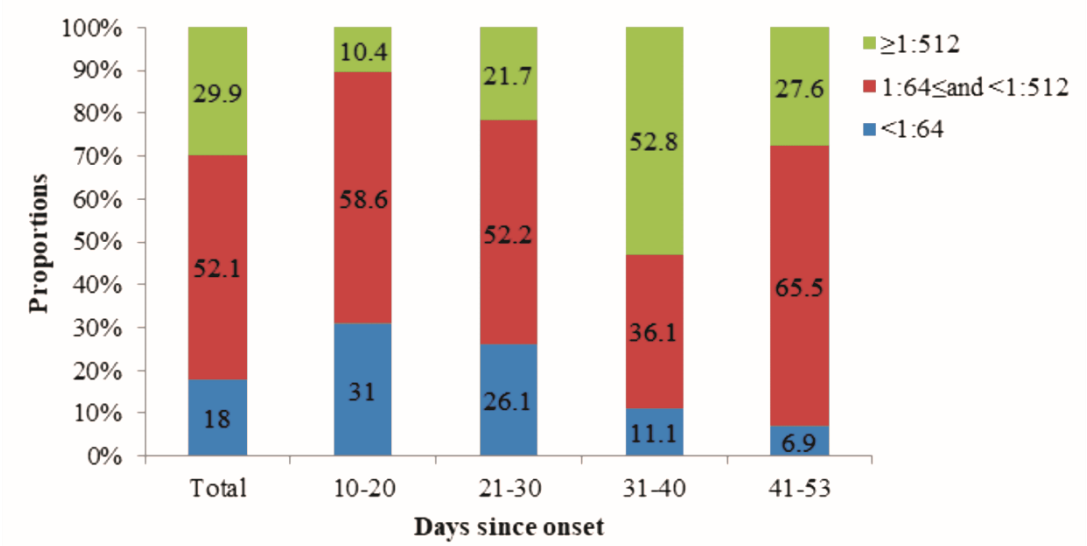
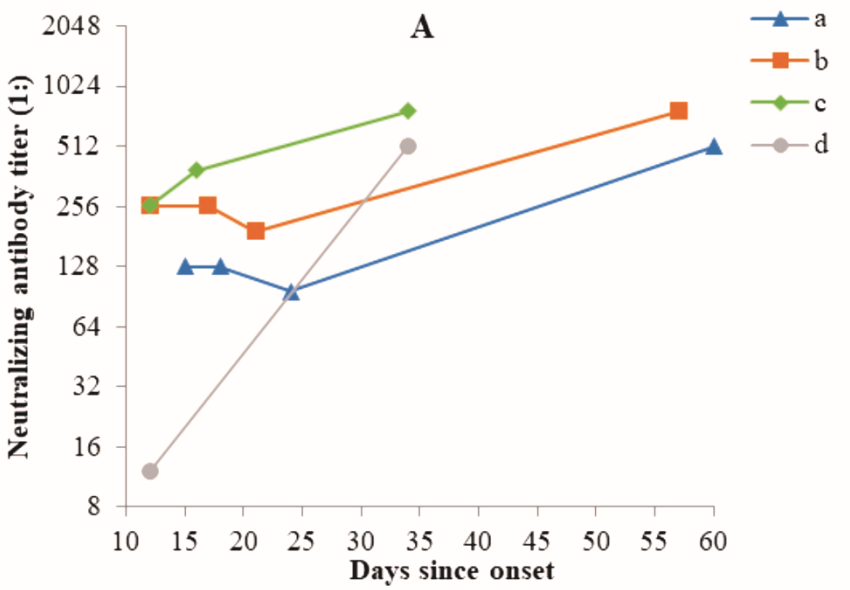
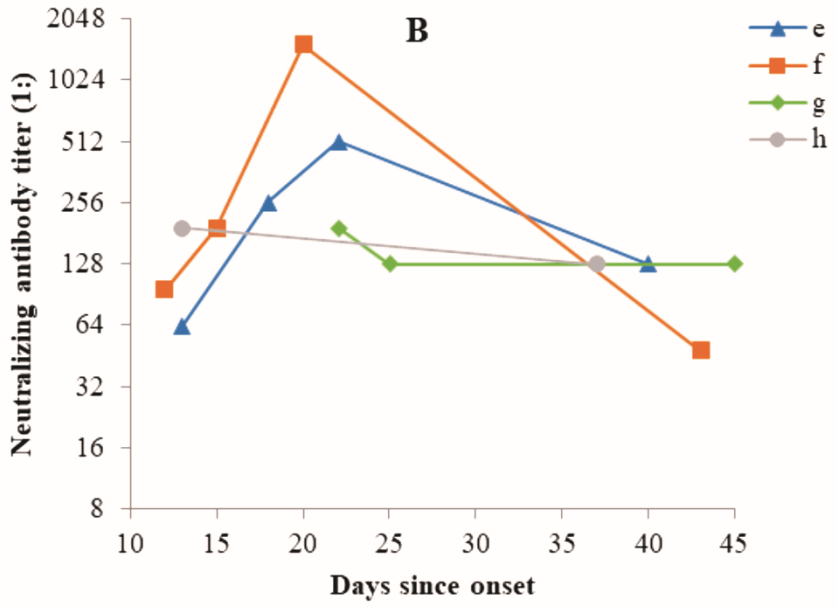


Figure 2


A
Neutralizing antibody titer (1:)
2048
1024
512
256
128
64
32
16
8
10
15
20
25
30
35
40
45
50
55
60
Days since onset
a
b
c
d


B
Neutralizing antibody titer (1:)
2048
1024
512
256
128
64
32
16
8
10
15
20
25
30
35
40
45
Days since onset
e
f
g
h