Available online at www.blood-genomics.com

Blood and Genomics

2021, 5(1): 41−47

B & G

Original Article

Comparative analysis of antibody responses to SARS-CoV-2 in various populations

Lei Liu[1,2△], Wanbing Liu[1△], Yan Liu[1,2], Guomei Kou[1], Yaqiong Zheng[1], Liping Cai[1], Shengdian Wang[3*], Shangen Zheng[1,2*]

[1] *Department of Transfusion Medicine, General Hospital of Central Theater Command of the PLA, Wuhan, Hubei 430070, China;*
[2] *Wuhan School of Clinical Medicine, Southern Medical University, Wuhan, Hubei 430070, China;*
[3] *CAS Key Laboratory of Infection and Immunity, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China.*

ABSTRACT

This paper aimed to analyze antibody responses to SARS-CoV-2 in various populations. Two hundred and six COVID-19 patients, 46 convalescent patients, and 270 healthy population were enrolled. Antibodies against nucleocapsid protein (N) and spike protein's receptor-binding domain (RBD), and neutralizing antibody were detected. The results demonstrated both anti-N and anti-RBD antibodies could be detected in about 80% of COVID-19 patients and 90% of convalescent patients, while no antibodies could be detected in some convalescents and patients even after 14 days post-onset of symptoms. The level of anti-RBD antibody strongly correlated with the neutralizing activity of sera from these two cohorts. The titer of neutralizing antibody was lower in convalescents than that in active COVID-19 patients. In addition, the titer of neutralizing antibody was less than 1:80 in none of the severe COVID-19 patients, 18.8% in non-severe COVID-19 patients, and 32.6% in convalescents. The study suggests that the level of anti-RBD antibody is closely related to neutralization activity in COVID-19 patients and convalescents. Some SARS-CoV-2-infected cases trigger a weak antiviral immune response, and the level of neutralizing antibody may have a faster decay rate.

Keywords: SARS-CoV-2, serological assay, nucleocapsid protein, receptor-binding domain, spike protein, neutralizing antibody

INTRODUCTION

The outbreak of coronavirus disease 2019 (COVID-19) has spread around the world and become a global pandemic. The etiological agent of COVID-19 was identified as a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). As of 16 April 2021, it had caused nearly 140 000 000 confirmed infections and 3 000 000 deaths worldwide. There is an urgent need for the development of appropriate tests to identify infected patients and to assess immunity against SARS-CoV-2. Sequencing of the viral genome has allowed for the rapid development of nucleic acid-based tests that have been widely used for the

*Corresponding to: Shangen Zheng, Department of Transfusion Medicine, General Hospital of Central Theater Command of the PLA, 627 Wuluo Rd, Wuhan, Hubei 430070, China. E-mail: sxkzsg@sina.com.; Shengdian Wang, CAS Key Laboratory of Infection and Immunity, Institute of Biophysics, Chinese Academy of Sciences, 15 Datun Rd, Beijing 100101, China. E-mail: sdwang@ibp.ac.cn.

△These authors contributed equally to this work.

Conflict of interests: The authors declared no conflict of interests.

https://doi.org/10.46701/BG.2021012021102

diagnosis of acute (current) SARS-CoV-2 infection. The measurement of anti-SARS-CoV-2 antibodies is helpful for detecting cases with negative nucleic acid-based test results and will become paramount for understanding the prevalence of immunity to SARS-CoV-2 in the later phases of pandemic control[1−3].

Many serological assays for the detection of antibodies against SARS-CoV-2 spike (S) protein, receptor-binding domain (RBD) or nucleocapsid (N) protein are now widely available. An important application of serological assays is to understand antibody responses relating to SARS-CoV-2 infection and vaccination. The kinetics and magnitude of antibody responses seem to correlate with the clinical severity of the disease[4−5]. Some asymptomatic and mild COVID-19 cases do not develop seroconversion[6−7]. In addition, immunogenic proteins of closely related human coronavirus (HCoV) may trigger cross-reactive antibodies in the host and cross-reactivity for serological SARS-CoV-2 antibody assays[5,8−9]. Antibody response to RBD is viral species-specific and shows neutralizing activity *in vitro*[5]. A recently published case series on plasma transfer from convalescent COVID-19 patients also demonstrated *in vivo* effects[10−11]. Neutralizing antibody has been identified by single-cell sequencing of RBD-binding B cells enriched from convalescent COVID-19 patients[12−13]. A correlation between spike/RBD antibody and SARS-CoV-2 neutralization has been reported in patients infected with SARS-CoV-2[5,14−15].

Here, we systemically investigated antibody responses to SARS-CoV-2 in COVID-19 inpatients and discharged convalescents in Wuhan during the lockdown. The study aimed to analyze how antibody responses vary across these populations and to provide information regarding immune protection from reinfection with SARS-CoV-2.

MATERIALS AND METHODS

Patients and samples

Plasma samples from two cohorts of patients were used in this study: (1) COVID-19 patients (*n*=206), who were tested positive for viral RNA using real-time RT-PCR assay on pharyngeal swab specimens and treated in the PLA General Hospital of Central Theater Command between February 6th and April 4th, 2020, were enrolled. Patients' general information (age, sex, vital signs, and coexisting disorders), clinical data, laboratory data, and radiological characteristics were extracted from electronic medical records. (2) Convalescent patients (*n*=46), who had been confirmed by viral nucleic acid testing and had been treated in several hospitals in Wuhan, were enrolled. The convalescent COVID-19 patients were dischargedfromthehospitalwhometthefollowingcriteria: ① their body temperature returned to normal for more than 3 days; ② there were significant improvements in their respiratory symptoms and lung imaging showed significant absorption of inflammation; ③ the nucleic acid test of respiratory pathogens was negative on two consecutive occasions (with at least 1 day interval). All the enrolled patients were long-term residents of Wuhan. The reference samples were collected from 270 healthy population. One sample was collected from each patient. The study was approved by the Hospital Ethics Committee and written informed consent was waived for emerging infectious diseases.

Real-time reverse transcription polymerase chain reaction (RT-PCR) assay

Pharyngeal swab specimens were collected from patients and placed into a collection tube with 200 μL of virus preservation solution. Total RNA was extracted using a respiratory sample RNA isolation kit (Shuoshi, Shanghai, China). Real-time RT-PCR was performed using a nucleic acid testing kit (Daan, Guangzhou, China) for SARS-CoV-2 detection as previously described[2]. The open reading frame 1ab (ORF1ab) and N protein were simultaneously selected as the two target genes. The human GAPDH gene was used as an internal control. The specific primers and probes set for ORF1ab and N protein were as follows: ORF1ab-forward primer 5′-ACCTTCTCTTGCCAC TGTAGC-3′; ORF1ab-reverse primer 5′-AGTATC AACCATATCCAACCATGTC-3′; and the probe 5′-FAM-ACGCATCACCCAACTAGCAGGCATAT-BHQ1-3′; N-forward primer 5′-TTCAAGAAATTC AACTCCAG-3′; N-reverse primer 5′-AGCAGCAA AGCAAGAGCAGCATC-3′; and the probe 5′-VIC-TCCTGCTAGAATGGCTGGCAATGGCG-BHQ1-3′. A cycle threshold value (CT-value) of ≤ 40 was defined as a positive test result, and a CT-value > 40 was defined as negative.

Enzyme-linked immunosorbent assay (ELISA)

Serological assay was performed using an Enzyme-Linked Immunosorbent Assay kit (Lizhu, Zhuhai, China), which was developed for detecting IgM or IgG antibody against SARS-CoV-2 N protein as previously described[2].

Chemiluminescence analysis

The IgM and total antibodies against receptor-binding domain (RBD) of the SARS-CoV-2 spike protein in serum samples were tested using chemiluminescence microparticle immunoassay

(CMIA). The CMIA reagents were supplied by Xiamen InnoDx Biotech Co., Ltd. China (Xiamen, China). RBD was expressed by mammalian cells and used to develop the serological assays. The CMIA for IgM antibody detection was based on μ-chain capture immunoassay (IgM-CMIA), while total antibody detection was based on double-antigen sandwich immunoassay (Ab-CMIA). CMIA measurement was conducted with an automatic analyzer Caris 200 (Xiamen UMIC Medical Instrument Co. Ltd. China), of which 200 tests per hour were possible. The IgM cutoff value and total antibodies were calculated according to the manufacturer's instructions. A test was determined as positive if the signal/cutoff (S/CO) ratio was ≥ 1.0. The antibody level was positively associated with the relative light unit (RLU), as detected by Caris 200 system, and was displayed using the S/CO value for each assay.

Neutralizing activity assay

The presence of neutralizing antibody was determined using a modified cytopathogenic assay[16]. Serum samples were inactivated at 56°C for 30 min and diluted serially with cell culture medium in two-fold steps. The diluted serums were mixed with a virus suspension of 100 $CCID_{50}$ 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. The cytopathic effect (CPE) of each well was recorded under microscopes, and the neutralizing titer was calculated by the dilution number of 50% protective condition. The neutralizing antibody titer was calculated by Reed-Muench method on day 5. A titer of 1 : 4 or higher indicated seropositivity.

Statistical analysis

Continuous variables were described as means and standard deviations or medians and interquartile ranges (IQR). Categorical variables were expressed as counts and percentages. Independent group *t* tests were applied to continuous variables that were normally distributed; otherwise, the Mann-Whitney test was used. Categorical variables were compared using Chi-Square test, while the Fisher exact test was used when data were limited. The correlations between neutralizing antibody titers and anti-RBD antibody titers or laboratory findings were analyzed by nonparametric Spearman correlation test. Statistical analyses were performed using Statistical Product and Service Solutions (SPSS version 22.0) software. $P<0.05$ was considered statistically significant.

RESULTS

Detection of antibodies against N protein and RBD of SARS-CoV-2

Firstly, serological assays for IgM and IgG against N protein were validated by ELISA, and simultaneously for IgM and total antibodies against RBD of S protein by chemiluminescence, in serum samples collected from 270 healthy population. No sample was identified as positive for anti-N or anti-RBD antibody. Next, the assays were conducted in serum samples collected from 206 hospitalized COVID-19 patients and 46 convalescent patients. In the cohorts of COVID-19 patients, 79.1% (163/206) of the samples had detectable anti-N and anti-RBD antibodies, while 11.7% (24/206) and 1.0% (2/206) of the samples only contained anti-RBD or anti-N antibodies respectively. Anti-N and anti-RBD antibodies were both negative in 8.3% (17/206) of the samples, most of which were collected within 10 days after onset of symptoms (***Fig. 1***). One possible explanation for the negative anti-N and anti-RBD antibodies is that the antibodies were not produced in the early stages of the disease. However, 4 samples without detectable antibodies were collected 15-24 days after onset. The seroconversion of anti-N and anti-RBD antibodies were highly consistent in the convalescents. There were also 4 samples without

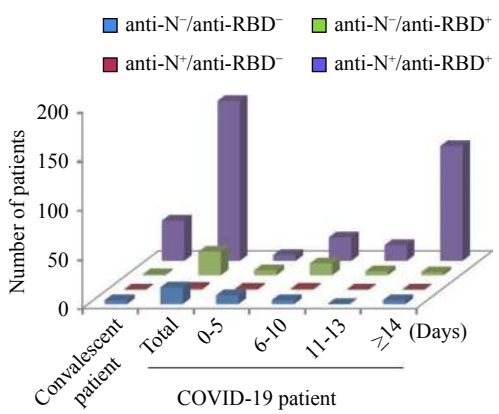

Fig. 1 **Distribution of anti-N and anti-RBD antibodies in 46 convalescent patients and 206 on course patients at different time since onset of symptoms.** Anti-N^+ and anti-RBD^+, anti-N^- and anti-RBD^+, anti-N^+ and anti-RBD^-, and anti-N^- and anti-RBD^- respectively represent dual positive of anti-N and anti-RBD antibodies, negative of anti-N antibodies and positive of anti-RBD antibodies, positive of anti-N antibodies and negative of anti-RBD antibodies, and dual negative of anti-N and anti-RBD antibodies.

detectable antibodies in the convalescent patients (***Fig. 1***). These data demonstrate that most patients infected with SARS-CoV-2 produce both anti-N and anti-RBD antibodies, but a few of them cannot produce detectable anti-SARS-CoV-2 antibodies.

Detection of neutralizing activity of plasmas

The neutralizing activity of serum samples was measured using a modified cytopathogenic assay on Vero cells. The specificity of the assay was validated with 50 randomly selected samples from 270 healthy population. No neutralizing activity was detected in these samples. Then, the neutralizing antibody was tested in 82 COVID-19 patients and 46 convalescents (***Table 1***). For the samples with detectable anti-N and anti-RBD antibodies, the positive rates of neutralizing antibody were both 100% in samples from COVID-19 patients (n=65) and convalescents (n=41). No neutralizing activity was detected in the samples from COVID-19 patients and convalescents who were negative for anti-N and anti-RBD antibodies. The levels of anti-RBD antibody were correlated with the samples' neutralizing activities. However, they were not entirely consistent. Most notably, neutralizing activity was very low or undetectable in some samples with high levels of anti-RBD antibody (***Fig. 2***). These data demonstrated that some infected people, including COVID-19 patients, might not acquire the immune protection against SARS-CoV-2, even if they produced a high level of antibodies.

Table 1 **The positive rates of neutralizing antibody**

Groups	No. of serum samples	Total	Anti-N^+ and anti-RBD^+	Anti-N^+ and anti-RBD^-	Anti-N^- and anti-RBD^+	Anti-N^- and anti-RBD^-
COVID-19 patients	82	71/82 (86.6)	65/65 (100.0)	0 (0.0)	6/7 (85.7)	0/10 (0.0)
Convalescents	46	42/46 (91.3)	41/41 (100.0)	0 (0.0)	1/1 (100.0)	0/4 (0.0)
Healthy population	50	0/50 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0/50 (0.0)

Anti-N^+ and anti-RBD^+ = dual positive of anti-N and anti-RBD antibodies. Anti-N^+ and anti-RBD^- = positive of anti-N antibodies and negative of anti-RBD antibodies. Anti-N^- and anti-RBD^+ = negative of anti-N antibodies and positive of anti-RBD antibodies. Anti-N^- and anti-RBD^- = dual negative of anti-N and anti-RBD antibodies.

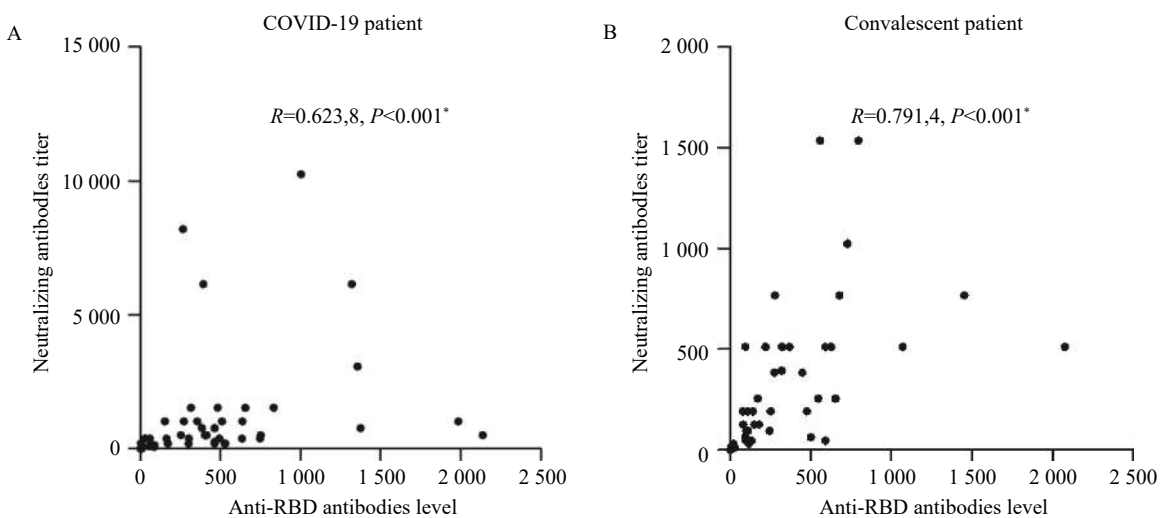

Fig. 2 **Correlation between the levels of anti-RBD antibodies and neutralizing antibody titers.** Scatter plots were generated using individual anti-RBD antibody level (x-axis) versus SARS-CoV-2 neutralizing antibody titers (y-axis) in COVID-19 patients (A) and convalescent patients (B). The nonparametric Spearman correlation coefficient (R) and the associated two-tailed P-value were calculated.

The associations of antibody levels with clinical classification

The relationships between antibody levels and disease characteristics were analyzed. In order to eliminate any interference with antibody production time, samples collected within 14 days after onset of symptoms were excluded. Consistent with previous research reports[4–5, 17], severe patients have higher levels of anti-RBD antibody and fewer lymphocytes in the peripheral blood than non-severe patients (***Fig. 3A***). However, there was no significant difference in neutralizing antibody levels between the severe and non-severe patients (***Fig. 4***). Neutralizing antibody levels were also not correlated with levels of

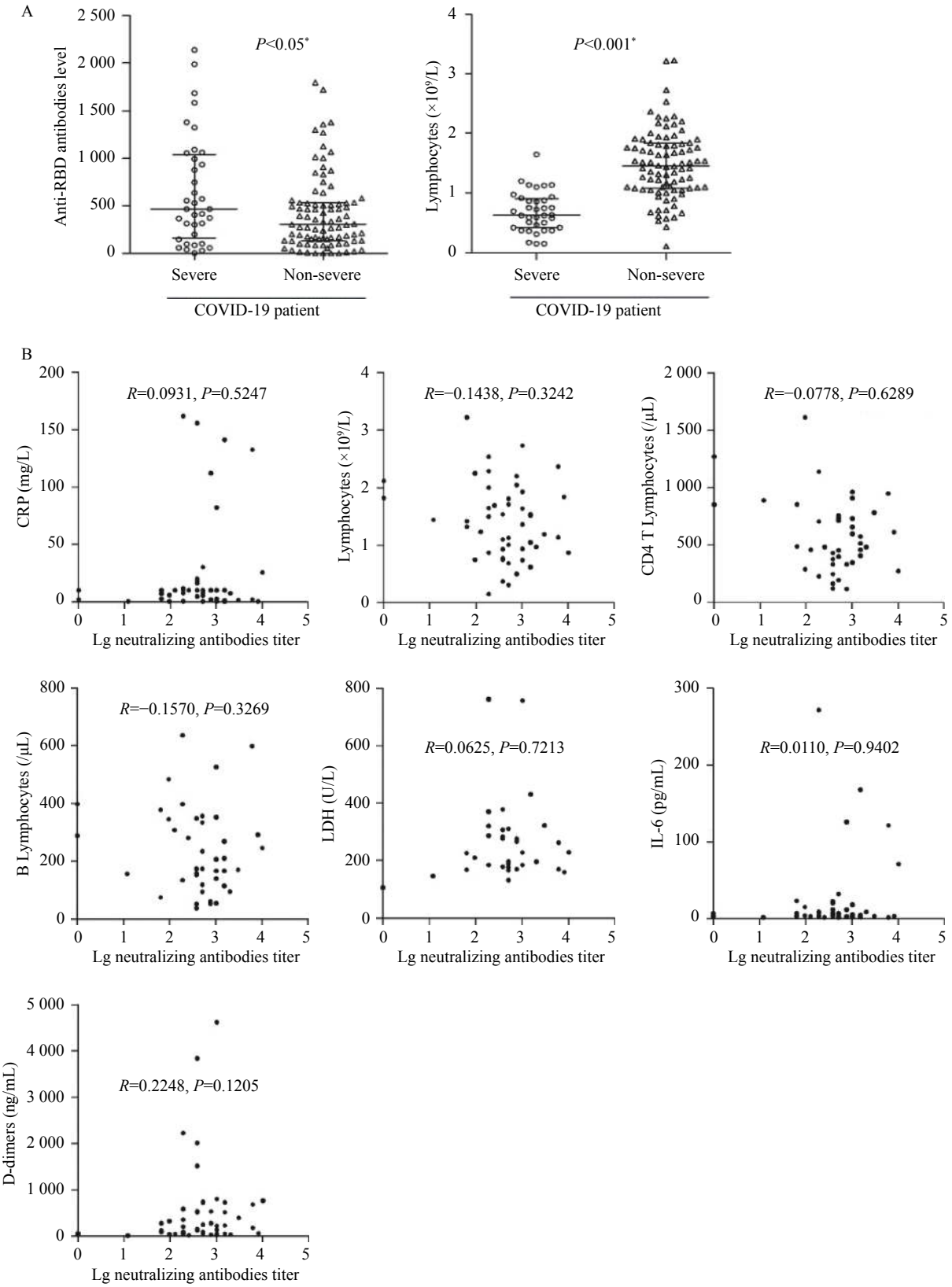

Fig. 3 **Associations between the antibody levels and clinical characteristics of the COVID-19 patients.** A. The anti-RBD antibody levels and lymphocytes were compared between the severe (circular) and non-severe (regular triangle) patients with COVID-19. Unpaired, two-sided Mann-Whitney U test *P* values were depicted in the plots, and the significant *P* value cutoff was set at 0.05. B. The correlation of the neutralizing antibody titers with laboratory findings (CRP, lymphocyte count, $CD4^+$ T cell count and B lymphocyte count, LDH, IL-6, D-dimers) were analyzed by nonparametric Spearman correlation test. Spearman correlation coefficients (R) and *P* value were depicted in plots.

IL-6, CRP, LDH and D-dimer (***Fig. 3B***).

In this study, all patients with severe disease had neutralizing titers of more than 1 ∶ 80. However, the neutralizing antibody titer was less than 1 ∶ 80 in 18.8% (6/32) patients with mild disease and 32.6% (15/46) convalescents (***Fig. 4***). All patients with severe disease had high levels of neutralizing antibody, which suggests that severe patients may have intensive viral infection and amplification which was able to trigger a strong antiviral immune response. Especially, the proportion of high antibody titers (≥1 ∶ 1 000) in convalescents was significantly lower than that in COVID-19 patients (***Fig. 4***). These data suggest that the level of neutralizing antibody against SARS-CoV-2 may have a fast decay rate.

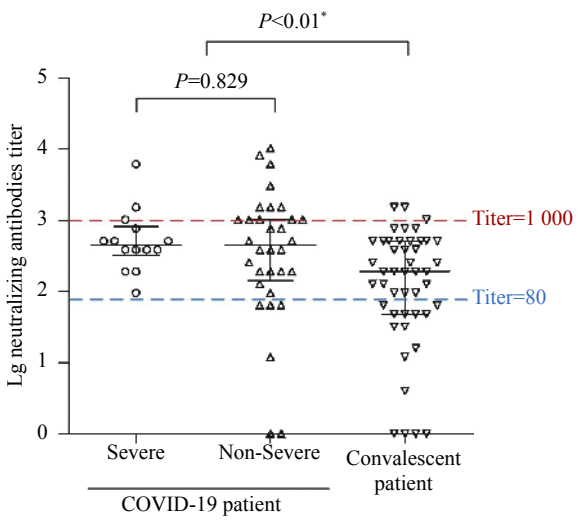

***Fig. 4* Comparison of neutralizing antibody titers in different populations.** Neutralizing antibody titers in severe (circular) and non-severe (regular triangle) COVID-19 patients, and convalescent patients (inverted triangle) were compared. Neutralizing antibody titers were expressed as a logarithm to base 10 on the y-axis. *P* values were calculated using Mann-Whitney U test.

DISCUSSION

Laboratory diagnostics of infected patients and the accurate assessment of immunity against SARS-CoV-2 present a major cornerstone in handling the current pandemic. Studies of antibody responses to COVID-19 have been reported in COVID-19 patients[1], convalescent patients[18−19], asymptomatic individuals infected with SARS-CoV-2[6] and the population without diagnosed infection[20]. However, how antibody responses vary across diverse populations with different co-morbidities, or infection histories is still not understood. In this study, we analyzed antibody IgM and IgG responses to N protein, IgM and total antibody responses to RBD of S protein, and the neutralizing antibody in hospitalized COVID-19 patients and discharged convalescents.

A growing number of *in vitro* diagnostic companies are developing SARS-CoV-2-specific antibody tests. Different viral antigens (RBD, N, S1) have already been evaluated in various proprietary and commercial kits. By measuring antibodies against N protein and RBD, as well as neutralizing antibody in the same serum samples, we found that the seropositivity of anti-N and anti-RBD antibodies were consistent in diagnosed cases with the COVID-19 disease. The RBD domain of S protein is critical for viral entry, thus the antibody targeting this domain of SARS-CoV-2 is expected to cast a better prediction for neutralizing antibody level and potentially protective immunity[9]. Consistent with other studies[5,14−15], we also observed a strong correlation between the levels of anti-RBD antibody and the neutralizing activity of sera from the COVID-19 patients and convalescents. However, some samples with high levels of anti-RBD antibody showed low neutralizing activity. This result suggests that testing the level of anti-RBD antibody does not reliably evaluate immune protection against the SARS-CoV-2 infection in individual cases.

The level and duration of neutralizing antibody after viral infection or vaccination are key issues for protection from (re-)infection. A recently published study involving asymptomatic individuals with lab-confirmed SARS-CoV-2 infection showed that the levels of IgG and neutralizing antibody significantly decreased within 2-3 months after infection[6]. In this study, the patients with severe disease were observed to be more likely to possess higher neutralizing antibody titer than mild disease and convalescents. These results indicate that some COVID-19 patients with mild symptoms cases may trigger a weak antiviral humoral immune response and decrease rapidly. Therefore, there are still urgent issues to be solved, such as how long immune protection can last and whether there is a risk of reinfection in patients (especially in asymptomatic and mild disease) with previous SARS-CoV-2 infection. Dynamic changes of antibody titer in infected individuals over time were not evaluated in this study. Further longitudinal serological studies with symptomatic and asymptomatic individuals are needed to determine the duration of protective immunity against SARS-CoV-2.

In conclusion, this study elaborated that nearly all patients with SARS-CoV-2 infection were able to produce anti-N and anti-RBD antibodies. The level of anti-RBD antibody titer had a close relationship with

SARS-CoV-2 neutralizing activity. However, the levels of neutralizing antibody especially in mild cases may have a faster decay rate.

Acknowledgments

This work was supported by the China Postdoctoral Science Foundation (2019M664008) and the Military Medical Science and Technology Youth Cultivation Project (20QNPY092). We thank all the patients involved in this study, as well as numerous doctors, nurses, and civilians working together to fight against the SARS-CoV-2. We thank Beijing Sinovac Biotech Ltd. for their support in neutralization assay. We appreciate the helpful advices from Prof. Sha Wu from Department of Immunology, School of Basic Medical Sciences, Southern Medical University and Prof. Lei Huang from Translational and Clinical Research Institute, Inflammation and Immunity Research Theme, Faculty of Medical Sciences, Framlington Place, Newcastle University, UK.

References

[1] Long QX, Liu BZ, Deng HJ, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19[J]. *Nat Med*, 2020, 26(6): 845−848.

[2] Liu L, Liu WB, Zheng YQ, et al. A preliminary study on serological assay for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in 238 admitted hospital patients[J]. *Microbes Infect*, 2020, 22(4-5): 206−211.

[3] Petherick A. Developing antibody tests for SARS-CoV-2[J]. *Lancet*, 2020, 395(10230): 1101−1102.

[4] Zhao JJ, Yuan Q, Wang HY, et al. Antibody responses to SARS-CoV-2 in patients with novel coronavirus disease 2019[J]. *Clin Infect Dis*, 2020, 71(16): 2027−2034.

[5] Okba NMA, Widjaja I, Li WT, et al. Serologic detection of middle east respiratory syndrome coronavirus functional antibodies[J]. *Emerg Infect Dis*, 2020, 26(5): 1024−1027.

[6] Long QX, Tang XJ, Shi QL, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections[J]. *Nat Med*, 2020, 26(8): 1200−1204.

[7] Zhang YC, Shen H, Wang XN, et al. Different longitudinal patterns of nucleic acid and serology testing results based on disease severity of COVID-19 patients[J]. *Emerg Microbes Infect*, 2020, 9(1): 833−836.

[8] McIntosh K, Kapikian AZ, Hardison KA, et al. Antigenic relationships among the coronaviruses of man and between human and animal coronaviruses[J]. *J Immunol*, 1969, 102(5): 1109-1118.

[9] Walls AC, Park YJ, Tortorici MA, et al. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein[J]. *Cell*, 2020, 181(2): 281−292. e6.

[10] Shen CG, Wang ZQ, Zhao F, et al. Treatment of 5 critically ill patients with COVID-19 with convalescent plasma[J]. *JAMA*, 2020, 323(16): 1582−1589.

[11] Salazar E, Perez KK, Ashraf M, et al. Treatment of coronavirus disease 2019 (COVID-19) patients with convalescent plasma[J]. *Am J Pathol*, 2020, 190(8): 1680−1690.

[12] Seydoux E, Homad LJ, MacCamy AJ, et al. Analysis of a SARS-CoV-2-infected individual reveals development of potent neutralizing antibodies with limited somatic mutation[J]. *Immunity*, 2020, 53(1): 98−105. e5.

[13] Cao YL, Su B, Guo XH, et al. Potent neutralizing antibodies against SARS-CoV-2 identified by high-throughput single-cell sequencing of convalescent patients' B cells[J]. *Cell*, 2020, 182(1): 73−84. e16.

[14] Amanat F, Stadlbauer D, Strohmeier S, et al. A serological assay to detect SARS-CoV-2 seroconversion in humans[J]. *Nat Med*, 2020, 26(7): 1033−1036.

[15] Premkumar L, Segovia-Chumbez B, Jadi R, et al. The receptor-binding domain of the viral spike protein is an immunodominant and highly specific target of antibodies in SARS-CoV-2 patients[J]. *Sci Immunol*, 2020, 5(48): eabc8413.

[16] Gao Q, Bao LL, Mao HY, et al. Development of an inactivated vaccine candidate for SARS-CoV-2[J]. *Science*, 2020, 369(6499): 77−81.

[17] Zhou F, Yu T, Du RH, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study[J]. *Lancet*, 2020, 395(10229): 1054−1062.

[18] Wang XL, Guo XH, Xin QQ, et al. Neutralizing antibody responses to severe acute respiratory syndrome coronavirus 2 in coronavirus disease 2019 inpatients and convalescent patients[J]. *Clin Infect Dis*, 2020, 71(10): 2688−2694.

[19] Robbiani DF, Gaebler C, Muecksch F, et al. Convergent antibody responses to SARS-CoV-2 in convalescent individuals[J]. *Nature*, 2020, 584(7821): 437−442.

[20] Xu X, Sun J, Nie S, et al. Seroprevalence of immunoglobulin M and G antibodies against SARS-CoV-2 in China[J]. *Nat Med*, 2020, 26(8): 1193−1195.

Received 20 January 2021, Revised 20 April 2021, Accepted 1 June 2021