SARS-CoV-2 Antibodies and Associated Factors at Different Hospitalization Time Points in 192 COVID-19 Cases

Jingyi Ou,[a,†] Mingkai Tan,[a,†] Haolan He,[a] Haiyan Tan,[b] Jiewen Mai,[a] Yaoxiang Long,[a] Xiaowen Jiang,[a] Qing He,[a] Ying Huang,[a] Yan Li,[a] Renshen Chen,[a] Liya Li,[a] Yaling Shi,[a,*,‡] and Fang Li[a,*,‡]

Background: We launched a retrospective analysis of SARS-CoV-2 antibodies in 192 patients with COVID-19, aiming to depict the kinetic profile of SARS-CoV-2 antibodies and explore the factors related to SARS-CoV-2 antibody expression.

Methods: Data on 192 confirmed patients with COVID-19 between January and February 2020 was collected from the designated hospital that received patients with COVID-19 in Guangzhou, China. Moreover, a cohort of 130 suspected patients with COVID-19 and 209 healthy people were also enrolled in this study. IgM and IgG antibodies to SARS-CoV-2 were detected by the chemiluminescence immunoassay kits in different groups.

Results: A total of 192 COVID-19 cases were analyzed, of which had 81.8% anti-SARS-CoV-2 IgM detected and 93.2% anti-SARS-CoV-2 IgG detected, respectively, at the time of sampling. The kinetics of anti-SARS-CoV-2 IgM and IgG showed that, the confirmed cases had anti-SARS-CoV-2 IgM seroconversion occurred 5–10 days after the onset of the symptoms, and then IgM rose rapidly to reach a peak within around 2–3 weeks, maintaining at its peak for 1 week before its decline. While they had anti-SARS-CoV-2 IgG seroconversion simultaneously or sequentially with IgM, reaching its peak within around 3 to 4 weeks and began to decline after the fifth week. Besides, correlation analysis showed that in patients with COVID-19 the level of IgM was related to gender and disease severity ($P < 0.01$), and the level of IgG was related to age and disease severity ($P < 0.001$). The univariate analysis of relevant factors indicated that the level of IgG had a weak correlation with age ($r = 0.374$, $P < 0.01$). The level of IgM in male patients was higher than that in female patients ($P < 0.001$). The expression level of anti-SARS-CoV-2 IgM and IgG were positively correlated with the severity of COVID-19 and the duration of the virus in the patients.

Conclusion: The findings of this study show that anti-SARS-CoV-2 IgM and IgG can be important assisting COVID-19 diagnosis, especially in the early phase of infection. Furthermore, antibody expression in patients with COVID-19 is also correlated with disease severity, age, gender, and virus clearance or continuous replication.

[a]Guangzhou Eighth People's Hospital, Guangzhou Medical University, Guangzhou, China; [b]Department of Laboratory Medicine, Guangzhou Baiyun District Maternal and Child Health Hospital, Guangzhou, China.
*Address correspondence to: YL.S at Department of Laboratory Medicine, Guangzhou Eighth People's Hospital, Huaying Rd., Guangzhou 510060, China. E-mail gz8hsyl@126.com. F.L. at Guangzhou Eighth People's Hospital, Huaying Rd., Guangzhou 510060, China. E-mail gz8hlf@126.com.
[†]Jingyi Ou and Mingkai Tan have contributed equally to this work.
[‡]These authors jointly supervised this work: Yaling Shi, Fang Li.
Received July 29, 2020; accepted January 4, 2021.
DOI: 10.1093/jalm/jfab003

IMPACT STATEMENT

The information about the kinetics of antibody (IgG and IgM) in the presented cases of our study will benefit the patients all over the world who are suffering SARS-CoV-2 infection. Our research data collected a cohort of 192 COVID-19 cases with different gender, ages, and disease severity, which could comprehensively characterize the issue. Meanwhile, there are a minority of systematic papers focusing on this field of study. Our study can help to promote serologically diagnosis, prediction of disease prognosis, and new vaccine development.

In December 2019, the outbreak of unexplained pneumonia happened in Wuhan, China. Later, the pathogen causing Coronavirus Disease 2019 (COVID-19) was found to be a novel coronavirus (SARS-CoV-2). As of May 18, 2020, 4.8 million confirmed cases and more than 310 000 deaths have been reported across the globe. The clinical manifestation of COVID-19 varies from no symptoms to severe pneumonia. Patients with severe disease usually have acute respiratory distress syndrome, respiratory failure, and they have to be monitored and treated in an Intensive Care Unit (ICU). The COVID-19 pandemic threatens public health and impacts the world economy.

Since the SARS-CoV-2 genome sequencing was completed in Wuhan (1), the real-time reverse transcription PCR (RT-PCR) assay has been broadly used as a "reference method" for COVID-19 diagnosis. Nevertheless, a survey from Ai et al. (2) suggested that the RT-PCR assay has a great possibility of false negative results, which depends on the sample types, the skill of sample collection, different stage of infection in patients, and the quality of the testing kits. Some researchers have found that the titer of SARS-CoV-2 antibodies was dynamically increased in the sera of patients with COVID-19 (3–5). Currently, diverse serologic testing kits have been developed. Chemiluminescence immunoassay has high sensitivity and specificity, a more stable detection effect, and has a lower biosafety risk than that of the RT-PCR assay for serum only used as sample. Here, we launched a follow-up analysis on SARS-CoV-2 antibodies in 192 patients with COVID-19, aiming to analyze the expression of SARS-CoV-2 antibodies against at different hospitalization time points and explore the factors related to SARS-CoV-2 antibody levels.

METHODS

Data Collection

A total of 192 patients with COVID-19 were enrolled in Guangzhou Eighth People's Hospital from January to February 2020. The diagnosis of COVID-19 was confirmed by RT-PCR assay. The disease severity varied from mild to severe. Based on the seventh edition of the Guidelines for Diagnosis and Treatment of Novel Coronavirus Pneumonia released by the National Health Commission of China, patients with severe COVID-19 had one of the following symptoms: (*a*) shortness of breath (respiratory rate $\geq$30 breaths per minute); (*b*) arterial oxygen saturation at resting state $\leq$93%; or (*c*) the ratio of the partial pressure of oxygen to fraction of inspired oxygen (PaO_2/FiO_2) $\leq$300 mmHg. Patients in a critical condition had at least 1 of the following manifestations: (*a*) respiratory failure requiring mechanical ventilation; (*b*) shock; and (*c*) multiple organ failure, or requiring monitoring and treatment in ICU. To evaluate the diagnostic effectiveness of the chemiluminescence kit, a cohort of

130 patients with suspected COVID-19 who had been recruited to the fever clinic or quarantine department of the hospital and finally had been excluded by the negative RT-PCR results were enrolled recruited [median age (IQR): 24(21–32) years; male: 77 cases]. From January 2019 to September 2019, a cohort of 209 healthy people undergoing regular physical examination in Guangzhou Baiyun District Maternal and Child Health Hospital were recruited as the control group. These healthy people were free of respiratory system infections, cardiovascular system diseases, hepatitis, immune system diseases, etc., with a median age of 49 (IQR, 32–56 years) and 100 cases were male. Informed consent was obtained from all patients, and the experimental protocol was approved by the Ethics Committee of Guangzhou Eighth People's Hospital (no. 20200547).

Detection of IgM and IgG to SARS-CoV-2 by Chemiluminescence Immunoassay

Using the separation gel vacuum tube, blood samples of approximately 3–5 mL were collected from individuals in different groups, followed by centrifugation at 1610*g* for 10 minutes to separate the sera. The serum IgM/IgG antibodies to COVID-19 were then tested on the MAGLUMI® 800 Chemiluminescent Analytical (CLIA) System purchased from Shenzhen New Industries Biomedical Engineering Co., according to the manufacturer's instructions. COVID-19 antibodies were captured by the recombinant SARS-CoV-2 nucleocapsid protein and spike protein, and *N*-(4-aminobutyl)-*N*-ethylisoluminol was used as the chemiluminescent marker, while horseradish peroxidase was the enzyme. The resultant chemiluminescent signals were measured as relative light units. To determine the results, a 2-point calibration curve between the chemiluminescence intensity and antibody relative concentration was established and the relative quantification of antibodies were presented as AU/mL. The cut-offs for SARS-CoV-2 IgM and IgG were set at 1.0 AU/mL. According to the manufacturer's manual, the sensitivities of the kit to IgM and IgG are 78.65 and 91.21%, respectively, and the specificities are 97.50 and 97.3%, respectively.

Statistical Analysis

Continuous variable data are demonstrated as mean ± standard deviation or median (interquartile range), while categorical variable data are expressed as frequencies and percentages. Parametric test (*t*-test) and nonparametric test (Mann–Whitney *U*-test) were used for comparisons of data with or without normal distribution, respectively. Chi-square test (χ^2 test) was used for categorical variable data analysis. Multivariate analysis of antibody levels was conducted using multiple linear regression equations. The relationship between the antibody level and age was analyzed by Pearson's correlation. A *P* value of less than 0.05 is regarded as statistically significant. Statistical analyses were performed by SPSS 22.0 (IBM SPSS) and GraphPad Prism 5.0 (GraphPad Software, Inc.).

DATA AVAILABILITY

The data are available from the corresponding author upon request.

RESULTS

Demographic and Clinical Characteristics of Patients with COVID-19

A total of 192 patients with confirmed COVID-19 were recruited in the study (median age = 52, IQR, 36–62 years), males accounted for 45.8% of the patients). 156 patients with mild or common symptoms were assigned to the nonsevere group, while 36 patients in a severe or critical condition were enrolled in the severe group. The baseline characteristics of patients in each group are

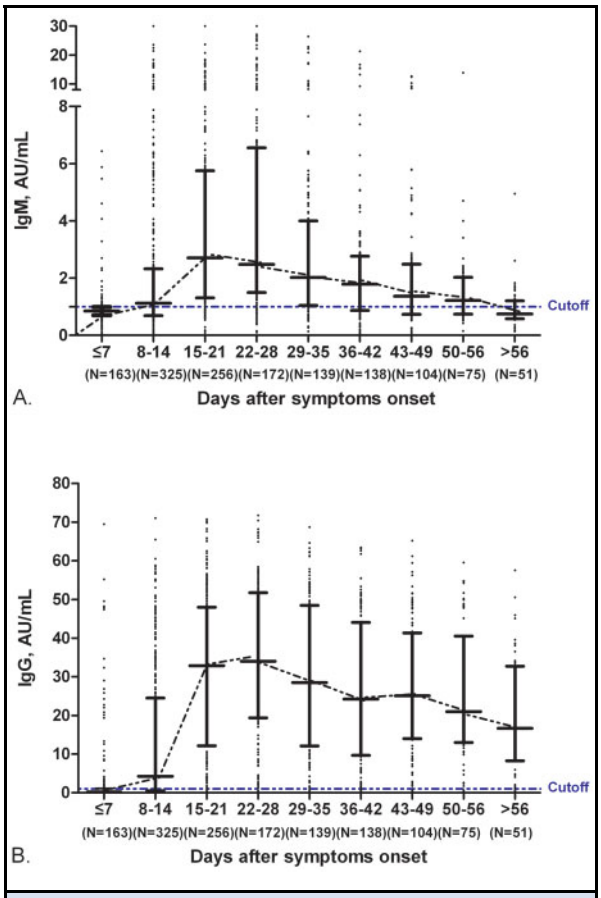

Fig. 1. (A) Kinetics of IgM and IgG to SARS-CoV-2 in patients with COVID-19. (B) Kinetics of IgM and IgG to SARS-CoV-2 in patients with COVID-19.

demonstrated in Supplemental Table 1. The median age of the severe group was older than that of the nonsevere group ($P < 0.001$), and the severe group had a longer hospital stay ($P < 0.001$). Male patients were more likely to deteriorate to severe or critical COVID-19 than female patients. 48.6% of patients with chronic diseases were in the severe group while 36.9% were in the nonsevere group. Nevertheless, the 2 groups showed no statistical difference in chronic diseases and body mass index (BMI). As of the date when the manuscript was completed, 4 patients still stayed in hospital because their chronic disease had not been effectively controlled. Their hospitalization has lasted for more than 110 days.

Performance of the Chemiluminescence Kit in SARS-CoV-2 Antibody Testing

The serological testing on 192 patients with COVID-19, 130 patients with suspected COVID-19 who were excluded from COVID-19 infection, and 209 healthy people was performed using the chemiluminescence kit. The rate of IgM positive tests (81.8%) was significantly lower than that of IgG positive tests (93.2%) in confirmed COVID-19 cases ($P < 0.001$). The false-positive rates of IgM and IgG in the negative control group were 2.4 and 1.9%. The chemiluminescence kit showed excellent low cross-reactivity ability in diagnosing COVID-19 as evidenced by the extremely low false-positive rate in the suspected COVID-19 group. The data are shown in Supplemental Table 2.

Kinetics of IgM and IgG to SARS-CoV-2 in Patients with COVID-19

To investigate the kinetics of IgM and IgG to SARS-CoV-2 in patients with COVID-19, 1423 specimens were collected from 192 patients with COVID-19 at different time points after disease onset. As shown in (Supplemental Table 3, Fig. 1) the seroconversion of SARS-CoV-2 IgM occurred 5–10 days after disease onset, and then IgM increased rapidly to reach a peak within around 2–3 weeks. The median peak level was 2.705 AU/mL. The high IgM expression was maintained for 1 week and then declined. Notably, IgM was not detected in one-third of the patients about 5 weeks after the onset of the symptoms, and was undetectable in more than half of the patients in around 8 weeks. COVID-19 patient had IgG seroconversion simultaneously with or after IgM seroconversion, reaching its peak in around 3–4 weeks. The median peak level of IgG was 33.998 AU/mL, which was higher than that of IgM. The IgG peak persisted for about 1–2 weeks and

then entered into a plateau or slowly decreased for as long as several weeks. SARS-CoV-2 IgG was detected at high level in 96% of patients about 8 weeks after the disease onset.

Additionally, we also discovered patients with delayed seroconversion. For example, a 27-year-old female patient (no. 95, shown in Fig. 2, L) who was IgG positive when returning to the hospital after hospital discharge for 33 days (56 days after the disease onset). She had no basic disease. IgM and IgG were undetectable in 13 patients (median age = 36, 5 males and 8 females), among whom 12 patients were tested during hospitalization and rehabilitation while 1 patient missed the tests during rehabilitation. All of them were nonsevere cases with an average period of 4 days between disease onset and hospital admission. Three of them had an asymptomatic infection (CT scans showed no pneumonia and no further nucleic acid detection after the first positive nucleic acid confirmatory test during hospitalization) and the other 10 were mild or common patients. A patient (no. 69 in Fig. 2, J) showed an upward trend of IgM expression, but the time point of IgM seroconversion was unsure due to the long interval between each test. Another patient (no. 78 in Fig. 2, K) received even less monitoring and tests so his data were incomplete. In these 2 patients, the negative antibody test results might not mean no antibodies were produced, and it was more likely that the best testing time was missed.

The Difference of SARS-CoV-2 Antibody Expression between the Negative Control Group and Confirmed COVID-19 Group

We selected the data of 3 tests, i.e., the first test, the last negative test, and the first positive test from confirmed cases. The data were compared with the data from the control group. We found that the antibody expression level in patients before the seroconversion was higher than that in the healthy population, with an upward trend. It suggests that the antibodies in patients with COVID-19 were produced early after the infection. However, the antibody level had not reached the detection threshold so there was no readout. Therefore, patients with suspected COVID-19 who have negative antibody test results should not be determined as uninfected. A series of serological tests should be conducted on patients with suspected COVID-19 to dynamically measure the antibodies to assist in the diagnosis.

To analyze the difference of expression level of antibody between patients with COVID-19 and the healthy population before seroconversion, 56 patients with COVID-19 receiving at least 2 serological tests before IgM and IgG seroconversion were selected. Then the data of 3 serological tests, i.e., the first test (around 4 days after onset), the last negative test (around 9 days after onset), and the first positive test (around 13 days after onset), were analyzed. The results showed that IgM levels in the last negative test were higher than the control group. Moreover, IgM in patients with COVID-19 started to increase at the early stage of the disease and the increase was more apparent than the increase of IgG. However, the level of IgG was significantly higher than that of IgM after seroconversion (Supplemental Table 4, Fig. 3).

SARS-CoV-2 Antibody-Related Factors in Patients with COVID-19

To screen SARS-CoV-2 antibody-related factors, multiple linear regression equations were used to analyze the correlations. Selected variables involved age, gender, disease severity, basic disease, and BMI. The results showed that in patients with COVID-19, IgM expression level was related to gender and disease severity ($P < 0.01$), and the level of IgG was related to age and disease severity ($P < 0.001$) (Supplemental Tables 5 and 6). The univariate analysis of relevant factors indicated that the level of IgG had a weak correlation with age ($r = 0.374$, $P < 0.01$) (Fig. 4). Interestingly, the level of IgM in male patients [(median: 3.054, IQR: 1.771–8.335) AU/mL)] was higher than that in

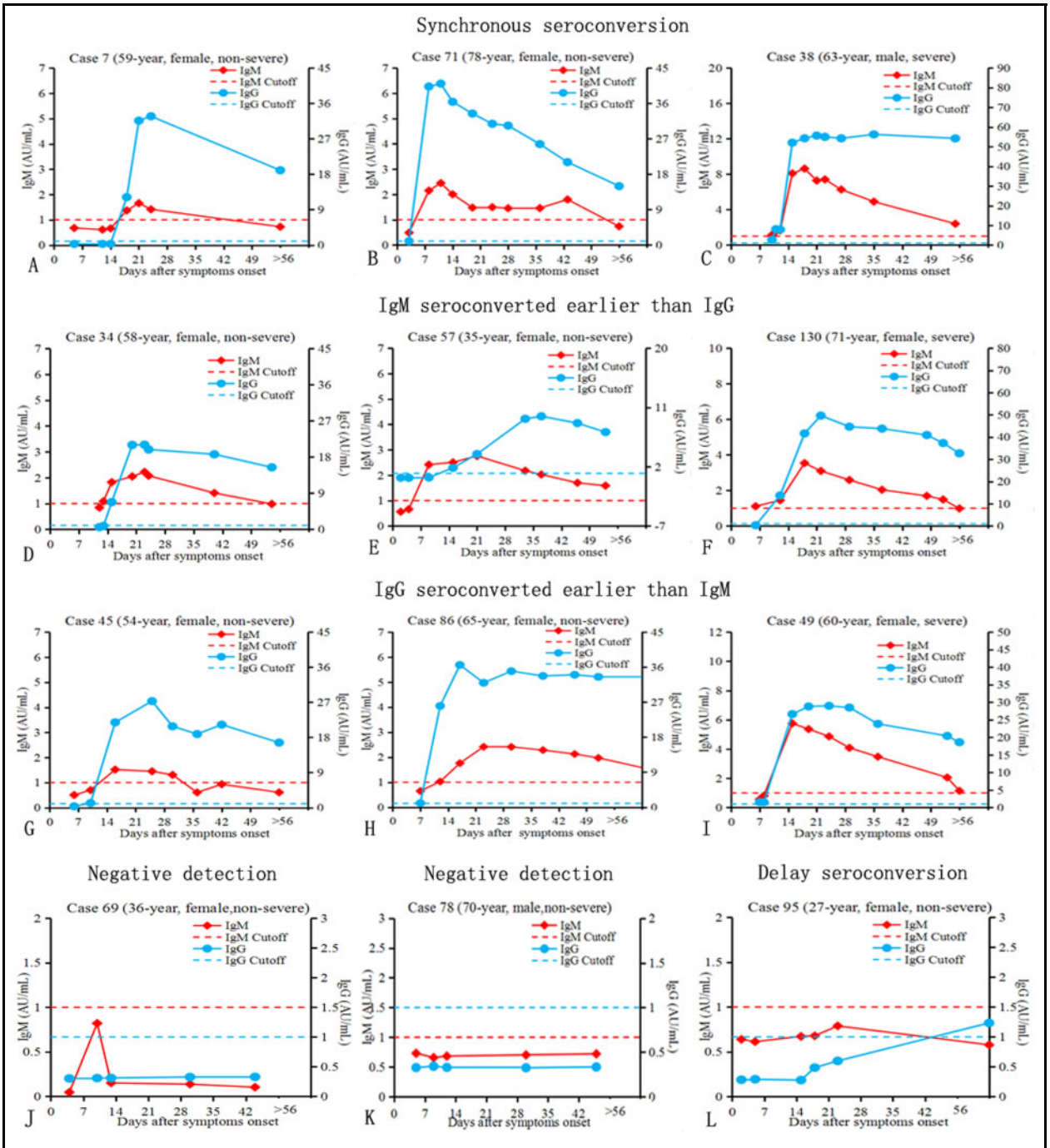

Fig. 2. Representative examples of different seroconversion types.

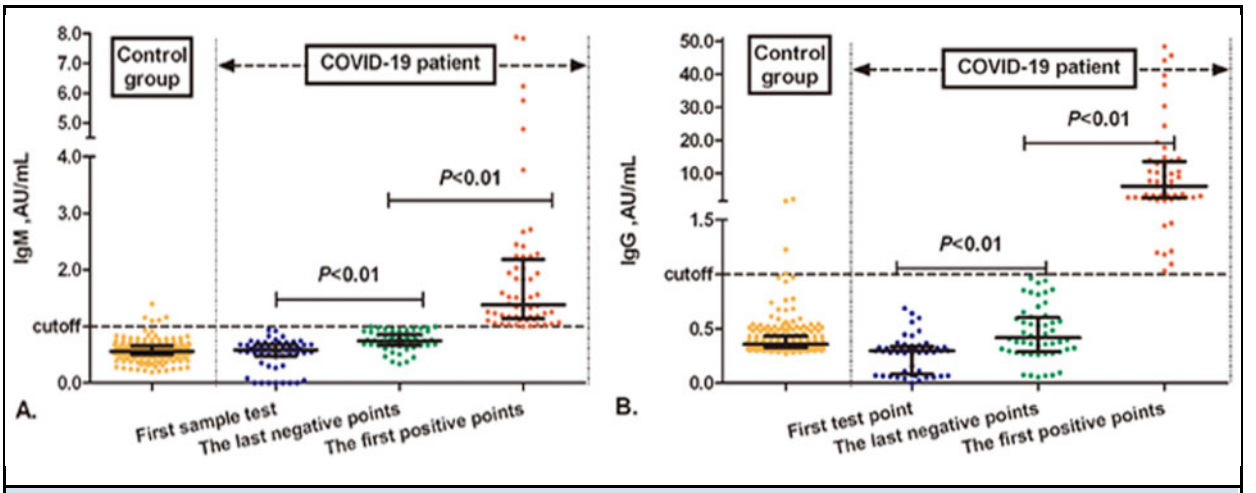

Fig. 3. SARS-CoV-2 antibody levels in the negative control group and confirmed COVID-19 group.

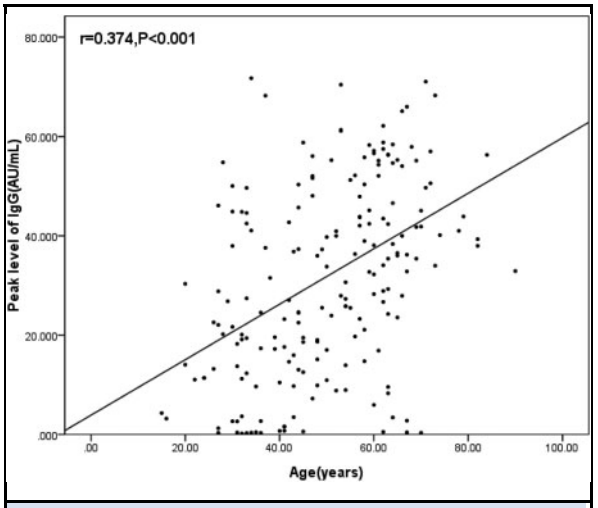

Fig. 4. The univariate analysis of relevant factors indicated that the level of IgG had a weak correlation with age (r = 0.374, P< 0.01).

female patients [(median: 1.743, IQR: 0.981–3.652) AU/mL] ($P < 0.001$) (Fig. 5). When comparing antibody levels in nonsevere and severe cases, we found that regardless of the hospitalization time, the levels of IgG and IgM in the severe group were always higher than those in the nonsevere group (Fig. 6).

The Continuous Results of RT-PCR and Serological Test in 4 Cases of Patients with COVID-19

From the nonsevere group and severe group, we respectively selected 2 patients who received continuous serological tests and RT-PCR assays in the whole disease process, aiming to explore the relationship between antibody expression and viral clearance or replication in patients with COVID-19. The median number of days of hospitalizations for the 4 patients was 29. Sputum or throat swabs were used for the molecular testing in the 4 cases. Detailed information on each case is shown in Supplemental Table 7a–d. We found that IgG expression in 2 nonsevere cases reached a peak on the 15th to 22nd day after the disease onset. The IgG levels ascended by more than 4 times and then gradually declined. Notably, RT-PCR results at this time point turned negative and remained negative until discharge. IgG expression in 2 patients with severe disease reached the peak (above 40 AU/mL) on the 15th to 17th days after the disease onset. In subsequent tests, patients who had persistently high levels of IgG, were receiving the persistently positive SARS-CoV-2 RNA test in their observation period.

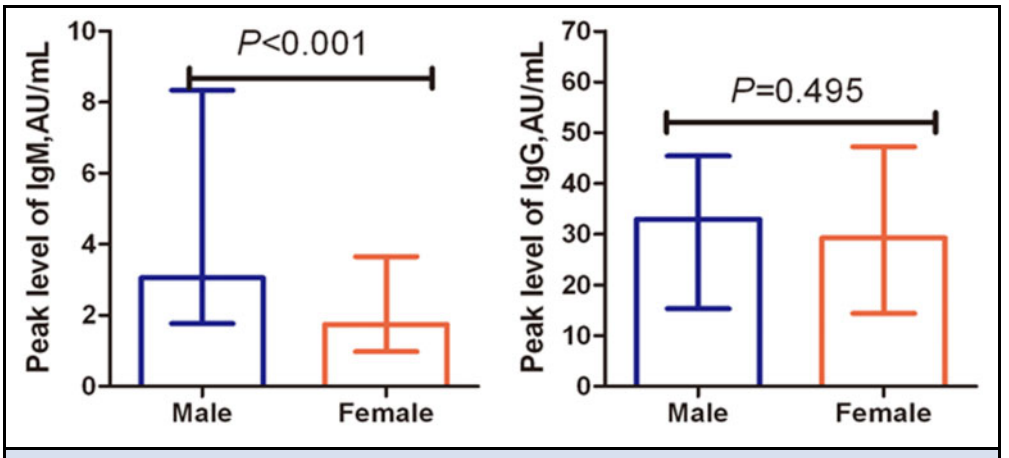

Fig. 5. SARS-CoV-2 antibody levels in different gender groups.

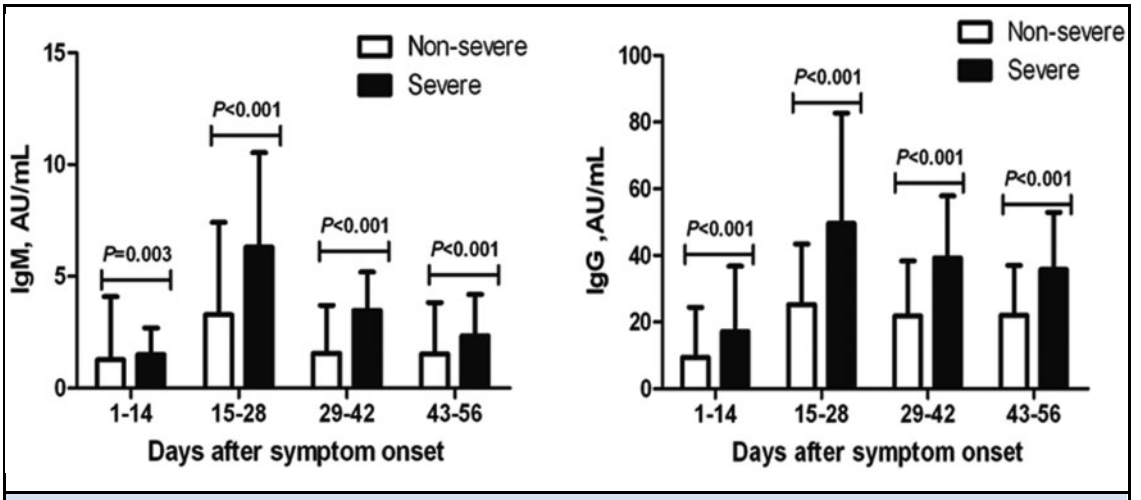

Fig. 6. Kinetics of SARS-CoV-2 IgM and IgG in patients with nonsevere and severe COVID-19.

DISCUSSION

The question about the immune responses in patients with COVID-19 is of particular concern to the world. To understand the dynamics of antibody production in COVID-19 and explore the factors related to antibody levels, we started to test patients with COVID-19 from day 2 after hospital admission.

The SARS-CoV-2 viral genome open reading frame contains the spike protein and nucleocapsid protein, which were considered as potential antigens for COVID-19 diagnostics (6, 7). To increase the detection efficiency of the chemiluminescence kit in this study, the recombinant antigen containing both the spike protein and nucleocapsid protein was used.

Since the false-positive rate and positive judgment value of this kit, which are stated by the manufacturer, may not apply to our research setting, we decided to first determine the actual positive judgment value with a large sample size to verify the sensitivity and specificity of the kit. We randomly selected 209 healthy participants as the reference control, and used the same batch of reagents to perform the tests. The upper limit of the 95% confidence interval of the luminescence value was compared with the positive interpretation value of the kit. Then the positive judgment value of the kit was determined. We figured out that the sensitivities of IgM or IgG were 81.8 and 93.2%, respectively, while the specificities of IgM and IgG were 97.6 and 98.1% respectively. These values were similar to the information provided by the manufacturer. The kinetics of anti-SARS-CoV-2 IgM and IgG showed that the confirmed cases had anti-SARS-CoV-2 IgM seroconversion.

Similar to the findings in previous reports (8, 9) in our study, patients with COVID-19 had IgG seroconversion earlier than IgM in 34%. We think that if this indeed happened, it poses a risk of misjudgment. We further consider that this phenomenon could be related to the low level and short duration of IgM. In addition, patients had spent a lot of time seeking medical assistance before hospital admission. Therefore, it was impossible to evaluate seroconversion before hospital admission. Due to the limitations of the study, it was extremely difficult for us to analyze blood samples every day and, unfortunately, the IgM seroconversion information was missed.

The first serological test was performed within 2 days of admission on each patient. We found that COVID-19 antibodies were produced early after the infection. However, the antibody expression levels were below the detection threshold so they were undetectable. Therefore, a patient with suspected COVID-19 whose antibody level was below the baseline cannot be regarded uninfected based on the result of only one test. The serological test on suspected patients with COVID-19 should be conducted multiple times to figure out the kinetics of antibody production.

Interestingly, IgM and IgG were not detected in 13 patients. Twelve of them were tested from hospitalization to rehabilitation period while one patient missed tests during rehabilitation. We speculate that perhaps the extremely low viral load or the fast viral clearance rendered low antibody levels and/or short antibody existence in these patients, making the antibody detection difficult.

In the study, we also hoped to understand the relevant factors of antibody production. Consistent with the results of former research, we found that patients with severe cases were older and stayed in the hospital longer. Their antibody levels were higher than those of the mild patients. The RT-PCR results in severe cases remained positive for a long time, while the IgG levels stayed high. The severity of viral infection depends on viral pathogenicity and host immunity. If the viral load is small or the virulence is weak, and the host has strong antiviral immunity, the host can quickly wipe out the virus through innate and adaptive immune reactions. We also found that IgM levels in male patients were higher than those in female patients, suggesting that distinct immunity between males and females would influence the disease progression. However, the relevant factors and mechanisms are far from being thoroughly understood, and further studies are still needed.

There were some notable limitations in our study. First, most patients in our study had mild symptoms and they were quickly discharged, hence we could not estimate the entire trend of the immune response in COVID-19 patient. Second, the data in this study were collected from Guanzhou District of China, which only represents certain regions. The humoral immune response pattern might be different in various

areas all over the world. However, we think that the expression level of antibody is universally positively correlated with the severity of COVID-19 and persistent existence of SARS-CoV-2 in hosts. Several questions are remaining unanswered: how long can SARS-CoV-2 IgG last in the human body? Can serum IgM and IgG in patients with COVID-19 be used as protective agents? Do patients with COVID-19 have secondary infections during the antibody-positive period after recovery? These questions are also the focus of our future investigations.

SUPPLEMENTAL MATERIAL

Supplemental material is available at *The Journal of Applied Laboratory Medicine* online.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.*

H. He, provision of study material or patients; F. Li, administrative support.

Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest*.

Employment or Leadership: None declared.
Consultant or Advisory Role: None declared.
Stock Ownership: None declared.
Honoraria: None declared.
Research Funding: Guangzhou Science and Technology Program (No. 202008010008), China.
Expert Testimony: None declared.
Patents: None declared.

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, preparation of manuscript, or final approval of manuscript.

Acknowledgments: We thank all health-care workers dedicated to the diagnosis and treatment of patients in Guangzhou Eighth People's Hospital.

REFERENCES

1. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet 2020;395:565–74.
2. Ai T, Yang Z, Hou H, Zhan C, Chen C, Lv W, et al. Correlation of chest CT and RT-PCR testing in coronavirus disease 2019 (COVID-19) in China: a report of 1014 cases. Radiology 2020;296:E32–E40.
3. Zhang W, Du RH, Li B, Zheng XS, Yang XL, Hu B, et al. Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. Emerg Microbes Infect 2020;9:386–9.
4. To KK, Tsang OT, Leung WS, Tam AR, Wu TC, Lung DC, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. Lancet Infect Dis 2020;20:565–74.
5. Padoan A, Cosma C, Sciacovelli L, Faggian D, Plebani M. Analytical performances of a chemiluminescence immunoassay for SARS-CoV-2 IgM/IgG and antibody kinetics. Clin Chem Lab Med 2020;58:1081–8.
6. Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y, et al. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease. Clin Infect DIS 2019;71:2027–2034.
7. Liu W, Liu L, Kou G, Zheng Y, Ding Y, Ni W, et al. Evaluation of nucleocapsid and spike protein-based ELISAs for detecting antibodies against SARS-CoV-2. J Clin Microbiol 2020;58:e00461–20.
8. Sun B, Feng Y, Mo X, Zheng P, Wang Q, Li P, et al. Kinetics of SARS-CoV-2 specific IgM and IgG responses in COVID-19 patients. Emerg Microbes Infect 2020;9:940–8.
9. Long QX, Liu BZ, Deng HJ, Wu GC, Deng K, Chen YK, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. Nat Med 2020;26:845–848.