Clinical Infectious Diseases

BRIEF REPORT

Profile of Immunoglobulin G and IgM Antibodies Against Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)

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

Keywords. SARS-CoV-2; COVID-19; serologic responses; IgG; IgM.

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

METHODS

Study Design

Between 11 January 2020 and 10 February 2020, 394 patients with COVID-19 were admitted to The Third People's Hospital of Shenzhen. SARS-CoV-2 was confirmed by 2 repeat positive results from our hospital and the local Chinese Centers for Disease Control and Prevention using 2 different commercial RT-PCR kits approved by the National Medical Products Administration, according to the manufacturer's protocol. Forty-one patients with preserved serial serum samples were included in this study. Patients were classified using the following criteria:

1. Mild cases: clinical symptoms were mild without manifestation of pneumonia on imaging
2. Moderate cases: fever, respiratory symptoms, and with radiological findings of pneumonia
3. Severe cases: meeting any one of the following criteria: respiratory distress, hypoxia (SpO_2 ≤93%), or abnormal blood gas analysis (PaO_2 <60 mmHg, $PaCO_2$ >50 mmHg)
4. Critical cases: meeting any one of the following criteria: respiratory failure requiring mechanical ventilation, shock, or other organ failure that requires intensive care unit care

Forty-one patients were then divided into 3 groups: mild and moderate (15 patients), severe (16 patients), and critical (10 patients). A total of 347 serum specimens from these patients (5–31 samples from each patient) were collected between 3 and 43 days of disease onset for routine clinical testing. Control sera were collected from 10 patients with influenza and 28 patients completing routine check-ups between 4 and 10 February 2020 at our hospital. The control sera were tested for the presence of immunoglobulin G (IgG) and IgM simultaneously with COVID-19 sera by the same method. The study was approved by the Institutional Review Board of The Third People's Hospital of Shenzhen (number 2020–0036).

Antibody Detection

IgG and IgM antibodies against SARS-CoV-2 were measured using iFlash-SARS-CoV-2 IgG/IgM chemiluminescent immunoassay kit (C86095G/C86095M; YHLO Biotech, Shenzhen).

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According to the instructions, the sensitivity and specificity of the kits were 90% and 95% for IgG and 80% and 95% for IgM. As a screening assay for COVID-19 diagnosis, combined nucleocapsid (N) protein and spike (S) glycoprotein were used as coated antigens to increase the sensitivity. The levels of IgG and IgM antibodies were positively correlated with the relative luminescence unit (RLU), and were calculated as arbitrary units per milliliter (AU/mL). Briefly, the serum samples of both healthy patients and patients with confirmed COVID-19 were tested. According to the receiver operating characteristic (ROC) curve, the corresponding concentration point of AUC (area under the ROC curve) greater than 0.9 was defined as the cutoff point, and the level of this point was defined as 10 AU/mL.

Data Analysis

Scatterplots were drawn to illustrate the cumulative proportion of patients with IgG and IgM antibodies, and the corresponding levels of IgG and IgM antibodies in 41 patients. LOWESS (locally weighted scatterplot smoothing) curves were fitted to display and compare the trends of antibody responses among groups. One-way analysis of variance was used to compare the levels of antibodies among groups. Paired *t* test was used to compare the seroconversion time for IgM and IgG antibodies in individual patients. GraphPad Prism 8 software was used for the construction of the charts and statistical analysis.

RESULTS

All controls enrolled in the study tested negative (Supplementary Table 4). Basic demographic characteristics of the study participants are described in Supplementary Table 1. The median age was 62.0 years (interquartile range, 42.0–66.0 years), 34.1% were male, 22% had at least 1 comorbidity, 51.2% had been to Wuhan city, and 21.9% had been to other cities in Hubei province. A total of 97.6% of patients (40/41) had positive IgG results and 87.8% of patients (36/41) had positive IgM results. Given the fact that most of the early cases went to the hospital late (~8 days after illness onset), whose first serum specimens were already positive with SARS-CoV-2 IgG or IgM, seroconversions of IgG and IgM antibodies against SARS-CoV-2 were only observed in 16 (39.0%) and 21 (51.2%) patients, respectively (Figure 1A and B). The median time of seroconversion for IgG was 11 days (8–16 days) and for IgM was 14 days (8–28 days) after disease onset. On an individual basis, the seroconversion

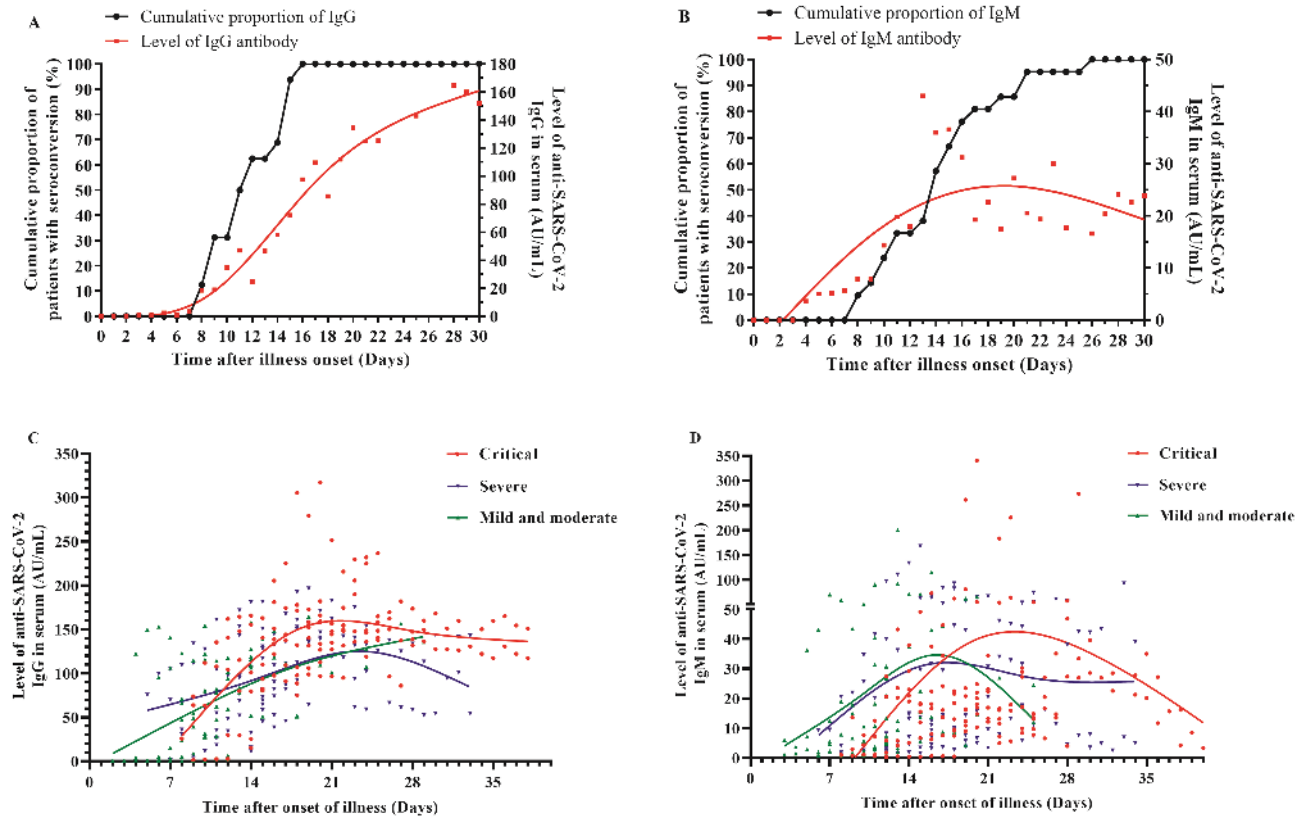

Figure 1. Longitudinal profile of IgG and IgM antibodies to SARS-CoV-2 nucleocapsid protein and spike glycoprotein in patients with COVID-19. *A*, Cumulative proportion of patients who seroconverted and the concentration level of anti–SARS-CoV-2 IgG in the sera of 16 patients. *B*, Cumulative proportion of patients who seroconverted and the concentration level of anti–SARS-CoV-2 IgM in the sera of 21 patients. *C*, The level (AU/mL) of anti–SARS-CoV-2 IgG in patients with mild and moderate, severe, and critical COVID-19 during hospitalization. *D*, The level (AU/mL) of anti–SARS-CoV-2 IgM in patients with mild and moderate, severe, and critical COVID-19 during hospitalization. Abbreviations: AU, arbitrary units; COVID-19, coronavirus disease 2019; Ig, immunoglobulin; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

time of IgG antibody was earlier than that of IgM antibody (12.45 ± 4.36 vs 13.75 ± 4.60 days, $P = .0019$) (Supplementary Table 2, Supplementary Figure 1). The level of IgG antibody reached the highest concentration on day 30, while the highest concentration of IgM antibody appeared on day 18 but then began to decline.

The trends in antibody production were analyzed among the 3 groups with different disease severities during the first 6 weeks after disease onset, as illustrated in Figure 1C and D. For IgG, the fitting curve of those in the critical group rose rapidly above the cutoff value from day 7 and peaked on day 20, while the fitting curves of the noncritical groups rose slightly from day 5. Although the IgG level of those in the mild and moderate group was still rising on day 28, the IgG response of the critical group was significantly stronger than that of noncritical groups within 4 weeks after illness onset ($P = .0001$) (Supplementary Table 3). For IgM, the fitting curve of the critical group rose above the cutoff value on day 10, peaked on day 23, then began to decline. However, the IgM levels of the noncritical groups rose above the cutoff value as early as day 5, peaked on day 16, and then decreased.

DISCUSSION

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

In the majority of the patients, there were antibody responses to SARS-CoV-2 during the first 3 weeks of the disease. The seroconversion time of IgG antibody was earlier than that of IgM antibody (Supplementary Table 2 and Supplementary Figure 1). The profile of antibodies against SARS-CoV-2 was comparable to previous findings of SARS-CoV infections [5, 6]. Li et al [5] reported that both IgG and IgM antibody levels increased to detectable levels from the second week of illness in 20 patients with SARS-CoV. Similarly, Woo et al [6] also observed that the seroconversion time for IgG was 3 days earlier than that for IgM after the SARS-CoV infection. The negative IgM results in 5 patients were possibly caused by the window phase of antibody production, as serum specimens were collected between day 3 and day 13; thus, longer surveillance is needed.

On the other hand, Park et al [7] reported that early antibody response was associated with reduced disease severity in MERS-CoV infections. Xu et al [8] revealed that an imbalance of the immune system was a pathogenetic factor from the pathological finding of a COVID-19 case. Here, compared with noncritical groups, we also observed delayed IgG and IgM antibody responses in the critical group (Figure 1C and D). Moreover, the slope of the IgG antibody response was steeper in the critical group (Supplementary Table 3), which might indicate an inflammatory storm. The intervention window might be the second week after illness onset for most patients.

Our study has several limitations. First, Liu et al [9] found that acute lung injury in Chinese macaques caused by SARS-CoV could be mediated by higher antispike IgG, and we detected high levels of IgG antibody in critical patients. Since we used combined N and S proteins as capture antigens to increase the sensitivity of this assay, further studies are needed to separate the effects of specific anti-N and anti-S antibodies. Second, we did not test the possible cross-reactivity of our in-house assay with common human coronaviruses (eg, hCoV-OC43 or others), MERS-CoV, or SARS-CoV. No SARS-CoV or MERS-CoV infections had been reported by any of the patients in the study, and the infection rate of common hCoV infections has been estimated to be as low as 0.8% in a previous study [10]. Thus, even if the cross-reactivity exists, it would have limited impact on the validity of these findings.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. J. Q. and L. L. were responsible for the study design, data interpretation, literature search, and writing of the manuscript. C. W., Xiaoyong L., and G. Z. performed the serological testing. Xiaohe L., Z. J., and Q. Z. were responsible for the clinical management, patient recruitment, and data collection. C. W., Z. J., Xiaohe L., and Q. Z. collected and analyzed the data.

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