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Original Paper

Long-term follow-up of production of IgM and IgG antibodies against SARS-CoV-2 among patients with COVID-19

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Abstract

The patients diagnosed with coronavirus disease 2019 (COVID-19) produce IgM and IgG antibodies against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). However, the frequency and duration of antibody production still need to be fully understood. In the present study, we investigated the duration of antibody production after SARS-CoV-2 infection. The patients diagnosed with COVID-19 were monitored over twelve months for the production of SARS-CoV-2 IgM and IgG antibodies, and the characteristics of these patients were examined. Forty-five patients diagnosed with COVID-19 were enrolled, and thirty-four patients were followed up until they tested negative for SARS-CoV-2 IgM and IgG antibodies or up to twelve months after the date of a negative SARS-CoV-2 polymerase chain reaction (PCR) result. The positivity rates of SARS-CoV-2 IgM and IgG antibodies were 27.3% and 68.2% when SARS-CoV-2 PCR was negative, 20.6% and 70.6% after one month, 8.8% and 52.9% after three months, and 0.0% and 14.7% after six months, respectively. Moreover, we compared patients with milder conditions who did not require oxygen administration with those with severe conditions which required oxygen administration. The positivity rate of SARS-CoV-2 IgG antibodies was significantly higher in patients with severe conditions than in those with milder conditions on the date of a negative SARS-CoV-2 PCR result and after one month and three months, but not after six months. Patients with more severe COVID-19 produced more SARS-CoV-2 IgG antibodies. Moreover, it is suggested that the duration of IgG antibody production is independent of COVID-19 severity.

Key words: COVID-19, SARS-CoV-2, IgG antibodies, IgM antibodies, ferritin

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Abbreviations: ACE2, angiotensin-converting enzyme 2; ADAM17, a disintegrin and metalloprotease 17; ARDS, acute respiratory distress syndrome; BMI, body mass index; COVID-19, coronavirus disease 2019; CRP, C-reactive protein; HbA1c, hemoglobin A1c; Ig, immunoglobulin; IL, interleukin; KL-6, Krebs von den Lungen-6; LDH, lactate dehydrogenase; RT-PCR, reverse transcription-polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SD, standard deviation; WHO, World Health Organization.

Introduction

In December 2019, coronavirus disease 2019 (COVID-19) originated in Wuhan City in China and spread rapidly worldwide. On March 11, 2020, the World Health Organization (WHO) declared it a pandemic. Since then, infection control measures have been implemented globally; however, the number of patients with COVID-19 has increased, and the global pandemic continues[1]. Several patients developed pneumonia and severe respiratory failure, resulting in death. In Wuhan, 42% of the patients diagnosed with COVID-19 had acute respiratory distress syndrome (ARDS), and 52% died[2]. COVID-19 is caused by a newly discovered virus: severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Different strains account for severe and less severe disease manifestations[3].

Several aggravating factors have been reported for COVID-19. SARS-CoV-2 infects all age groups[4]; however, younger people exhibit mild conditions, whereas older people show severe conditions[5]. It is speculated that aging reduces lung function and delays the adaptive immune response, leading to the proliferation of the virus, which worsens inflammation and increases the risk of death. Additionally, males are more susceptible to COVID-19[6,7] because men are more susceptible to SARS-CoV-2 infection than women. The abundant disintegrin and metalloprotease 17 (ADAM17) expression in the lungs and liver contribute to SARS-CoV-2 infection. ADAM17 induces shedding of the angiotensin-converting enzyme 2 (ACE2) receptor, suppressing SARS-CoV-2 infection[8]. It is known that estradiol, which is abundant in women, increases the expression of ADAM17, resulting in a reduction in the number of ACE2 receptors. SARS-CoV-2 proliferates by binding to the ACE2 receptor and invading cells[9]; thus, men are more likely to be infected by the virus than women.

Upon blood examination, the high ferritin concentrations decreased lymphocyte counts, and increased interleukin 6 (IL-6) levels have been indicated as risk factors[5]. It is speculated that the decrease in lymphocyte count occurs because the cytoplasmic components of lymphocytes are damaged when SARS-CoV-2 invades target cells[10]. This was further observed in patients with severe middle east respiratory syndrome[11,12]. Additionally, C-reactive protein (CRP), IL-6, and interleukin 10 (IL-10) concentrations increase in patients with COVID-19[13–15]. Han *et al.* reported that patients with COVID-19 had twice the concentration of IL-6 and a 37% increase in IL-10, which increased more in patients with severe disease than in healthy controls[15]. IL-6 is a biomarker of the early stages of lung injury and is involved in the host's defense against infection and tissue damage[16]. In COVID-19, infection by SARS-CoV-2 induces the production of IL-6, causing a cytokine storm associated with systemic inflammation[17,18].

Investigations for SARS-CoV-2 infection include genetic tests, such as reverse transcription-polymerase chain reaction (RT-PCR), and immunological assays targeting the antigen or antibody[19,20]. The RT-PCR test detects SARS-CoV-2 in nasopharyngeal swabs and saliva and is widely used to diagnose COVID-19[20,21]. The immunological antigen test can detect SARS-CoV-2 antigens in nasopharyngeal swabs and diagnose COVID-19. This method can be performed more efficiently than RT-PCR, and the results are obtained more quickly, which is suitable for screening tests; however, the sensitivity is slightly inferior to that of RT-PCR[22].

The SARS-CoV-2 antibody test is a blood test for detecting antibodies against SARS-CoV-2. The specific immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies against SARS-CoV-2 are mainly measured as antibody titers[23]. Haveri *et al.* reported that SARS-CoV-2 IgM and IgG antibodies were detected nine days after the onset of symptoms but were not detected on Day 4[24]. They also showed that the IgG antibody titer increased until Day 20. In addition, Li *et al.* reported that when SARS-CoV-2 IgM or IgG antibodies were detected, the sensitivity for infection was 88.7%, and the specificity was 90.6%[25]. Moreover, SARS-CoV-2 IgG antibodies may neutralize the virus[26]. Based on these results, it is crucial to ascertain the periods of maturation and deployment of SARS-CoV-2 IgM and IgG antibodies to prevent and treat COVID-19. Currently, the production of SARS-CoV-2 IgM and IgG antibodies is being verified; however, the timing and progression of antibody production still need to be clarified.

We investigated the expression of SARS-CoV-2 IgM and IgG antibodies in patients with COVID-19 over twelve months to clarify the progression and characteristics of antibody production. Furthermore, the relationships between antibody production and aggravating factors were examined.

Material and methods

1. Patient Enrollment

The patients hospitalized with a diagnosis of COVID-19 based on SARS-CoV-2 PCR positivity were recruited for this study. Recruitment was conducted from March 1 to August 31, 2020, at the Showa University Hospital, Showa University Koto Toyosu Hospital, Showa University Fujigaoka Hospital, and Showa University Northern Yokohama Hospital. Permission to participate in this study was obtained from the patients when SARS-CoV-2 PCR turned negative. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and / or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. The study design was approved by the ethics committee of Showa University, Tokyo, Japan (approval number : 3153). Written informed consent was obtained from all enrolled patients.

2. Parameters for Investigation

The patients' backgrounds were investigated during admission, and blood tests were performed. Subsequently, blood tests were performed at the time of confirmation of negative PCR and one, three, six, nine, and twelve months after negative confirmation. Blood tests assessed the SARS-CoV-2 IgM and IgG antibodies, white blood cell count, percentage of lymphocytes, D-dimer, CRP, ferritin, lactate dehydrogenase (LDH), Krebs von den Lungen-6 (KL-6), and hemoglobin A1c (HbA1c). Based on the WHO COVID-19 guidelines, the group that did not require oxygen administration was categorized as the non-severe group, and the group that required oxygen administration was categorized as the severe group[27].

3. Methods for testing SARS-CoV-2 IgM and IgG antibodies

The collected blood was centrifuged, and the serum was collected. The antibody test was performed using a SARS-CoV-2 IgM / IgG Antibody Test Kit (GenBody Inc., Chungcheongnam-do, South Korea). After adding 10 μl of serum to the antibody test kit and allowing it to stand for 15 min, positivity was characterized by both the positive control line and the test lines for SARS-CoV-2 IgM / IgG antibodies turning red.

4. Statistical Analysis

The two groups were compared using an unpaired Student's *t*-test. The chi-squared test was used to compare the proportion of SARS-CoV-2 IgM and IgG antibody positivity in the non-severe and severe groups. The Mann-Whitney *U* test was used to compare the continuous data of the non-severe and severe groups. The correlations between the biomarkers and the date of negativity for SARS-CoV-2 IgG antibodies were assessed using the Pearson correlation coefficient. Analyses were performed using JMP® Pro 16 software (SAS Institute Japan, Tokyo, Japan). The data are expressed as mean ± standard deviation (SD), and statistical significance was set at $P<0.05$.

5. English proofreading

English proofreading was performed by Editage (www.ediitage.com).

Results

1. Patient Characteristics

Forty-five patients met the inclusion criteria and were enrolled in the study. After initiating the investigation, thirty-four patients were followed up for twelve months after SARS-CoV-2 PCR was negative or until SARS-CoV-2 IgM and IgG antibody tests were negative. Eleven patients did not attend outpatient visits ; hence, they were excluded from the study. The mean age of the patients was 54.3 ± 13.2 years, 82.4% were male, and the mean body mass index (BMI) was 24.6 ± 4.03 kg/m^2 (Table 1). There were fifteen and nineteen patients in the non-severe and severe groups. The mean age of patients in the non-severe group was 44.1 ± 11.6 years, and that of those in the severe group was 62.4 ± 7.40 years, suggesting that patients in the severe group were significantly older ($P<0.001$). Moreover, the mean number of days from the onset of symptoms to SARS-CoV-2 PCR negativity was 9.67 ± 5.93 days for the non-severe group and 18.8 ± 10.3 days for the severe group, suggesting that the severe group took significantly more days to attain PCR negativity ($P=0.004$).

2. Relationship between COVID-19 severity and blood examination

The results of blood examinations of the non-severe and severe groups were compared (Table 2). Significant differences were observed in the percentage of lymphocytes and the concentrations of

Table 1. Patient characteristics

	all (n=34)	non-severe (n=15)	severe (n=19)	*P* value
Age, yr	54.3 ± 13.2	44.1 ± 11.6	62.4 ± 7.40	<0.001
Sex, F (%)	6 (17.6)	4 (26.7)	2 (10.5)	NS
height, cm	170.0 ± 7.8	168.0 ± 8.6	170.8 ± 6.9	NS
weight, kg	71.4 ± 15.5	70.9 ± 16.6	71.8 ± 14.6	NS
BMI, kg/m^2	24.6 ± 4.03	24.8 ± 4.09	24.5 ± 3.99	0.80
Duration, days	14.8 ± 9.76	9.67 ± 5.93	18.8 ± 10.3	0.004

Values are means ± SD or n (%). Duration: The number of days until PCR becomes negative. NS, not significant; BMI, body mass index; SD, standard deviation, PCR, polymerase chain reaction.

Table 2. Blood examinations

	all (n=34)	non-severe (n=15)	severe (n=19)	*P* value
WBC, /ml	5,990 ± 2,400	5,490 ± 1,240	6,370 ± 2,990	0.27
Neu %	73.1 ± 9.69	67.0 ± 8.22	80.1 ± 5.72	<0.001
Lym %	18.7 ± 8.32	23.8 ± 6.86	12.8 ± 5.45	<0.001
D-dimer, mg/ml	6.26 ± 26.7	0.859 ± 0.422	10.5 ± 35.1	0.26
CRP mg/dl	9.01 ± 10.7	1.33 ± 1.43	15.1 ± 10.9	<0.001
Ferritin, ng/ml	1,235 ± 1,712	346 ± 199	2,124 ± 2,060	0.008
LDH U/l	355 ± 218	193 ± 36.6	517 ± 203	<0.001
KL-6.ng/ml	326 ± 231	237 ± 120	394 ± 270	0.04
BNP pg/ml	17.3 ± 23.4	10.6 ± 9.10	24.0 ± 30.4	0.17
HbA1c %	6.10 ± 0.844	5.55 ± 0.410	6.61 ± 0.825	0.002

The values are mean ± SD. WBC, white blood cell; Neu, neutrophil; Lym, lymphocyte; CRP, c-reactive protein; LDH, lactate dehydrogenase; KL-6, Krebs von den Lugen-6; BNP, brain natriuretic peptide; HbA1c, hemoglobin A1c; SD, standard deviation.

CRP, ferritin, LDH, KL-6, and HbA1c. Differences in the percentage of lymphocytes, ferritin concentration, and HbA1c level between the non-severe and severe groups were significant (23.8 ± 6.86% vs. 12.8 ± 5.45%, $P<0.001$; 346 ± 199 ng/ml vs. 2,124 ± 2,060 ng/ml, $P=0.008$; and 5.55 ± 0.410% vs. 6.61 ± 0.825%, $P=0.002$, respectively).

3. Investigation of SARS-CoV-2 IgM and IgG antibodies

We investigated SARS-CoV-2 IgM and IgG antibody positivity rates and the duration for attaining negativity. Positivity was more likely for SARS-CoV-2 IgG antibodies than IgM antibodies over the entire study period (Figure 1). The SARS-CoV-2 IgM antibody positivity rate at the point of SARS-CoV-2 PCR negativity was 27.3%. The percentage of IgM antibody-positive samples was 20.6%, 8.8%, and 0.0% after one, three, and six months, respectively, after the date of PCR negativity. SARS-CoV-2 IgG antibody positivity rate at the point of SARS-

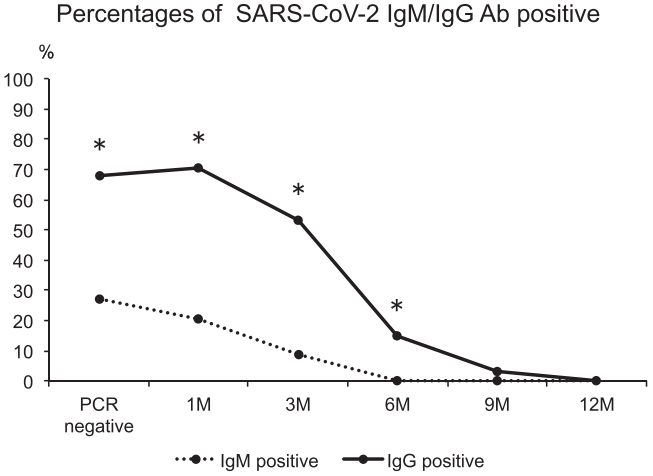

Fig. 1. Rate of positivity for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) IgM and IgG antibodies at the point of testing negative for SARS-CoV-2 by polymerase chain reaction and one, three, nine, and twelve months later. *$P<0.05$ (n=34).

CoV-2 PCR negativity was 68.2%. The rate of IgG antibody positivity was 70.6%, 52.9%, 14.7%, 2.9%, and 0.0% after one, three, six, nine, and twelve months, respectively, after the date of PCR negativity (Figure 1). The positive rates of SARS-CoV-2 IgM antibodies at the point of SARS-CoV-2 PCR negativity for patients in the non-severe and severe groups were 16.7% and 40.0%, respectively (Figure 2a). The rates of IgM antibody positivity for the non-severe and severe groups were 6.7% and 31.6% after one month, 6.7% and 10.5% after three months, and 0.0% after six months, respectively. There was no statistically significant difference in the rate of IgM antibody positivity over the entire study period (Figure 2a). When the rates of IgG antibody positivity for the non-severe and severe groups were compared, the rate at the time of PCR negativity was 41.7% and 100.0%, respectively (Figure 2b). The rates of IgG antibody positivity for the non-severe and severe groups were 40.0% and 94.7% after one month, 40.0% and 63.2% after three months, 13.3% and 15.8% after six months, 6.7% and 0.0% after nine months, and 0.0% for both groups after twelve months, respectively. The IgG antibody concentration was significantly higher in patients in the severe group after one month ($P<0.001$); however, no significant difference was observed three months after the PCR was negative (Figure 2b).

Moreover, we investigated the correlations between the biomarkers from the blood examination and the date of negativity for SARS-CoV-2 IgG antibody testing (Figure 3). The inflammation-related markers, CRP and ferritin, significantly correlated with the negative testing time for IgG antibodies (Figures 3a, b). The time elapsed before testing negative for IgG antibodies showed no correlation with the percentage of lymphocytes and the concentration of KL-6 (Figures 3c, d) and a significant correlation with LDH concentration (Figure 3e). Additionally, HbA1c concentration correlated with the time elapsed until testing negative for IgG antibodies (Figure 3f).

Discussion

Here, we investigated the presence of SARS-CoV-2 IgM and IgG antibodies in COVID-19 patients over twelve months in the Japanese population. Analyses of the blood of the patients with COVID-19 showed that ferritin, IL-6, and D-dimer concentrations are higher in non-survivors than in survivors[5]. Hou *et al*. reported that the serum concentrations of CRP, ferritin, and IL-6 significantly increase with the severity of illness in patients with COVID-19[28]. The serum concentration of ferritin increases with worsening COVID-19. Therefore, it could be used to identify patients at risk of aggravation or death[5]. In this study, we found that the ferritin concentration on admission was higher in patients with severe disease than those without severe disease, consistent with previous reports. Furthermore, we found that the production of SARS-CoV-2 IgG antibodies was prolonged with a higher ferritin concentration. These results suggest that ferritin concentration could be used to predict aggravation and the duration of neutralizing antibody production.

The SARS-CoV-2 IgG antibody positivity rate was higher than that of IgM antibody positivity during the early stages of COVID-19. Six months after SARS-CoV-2 PCR negativity, all patients tested negative for SARS-CoV-2 IgM antibodies, and 80% of patients

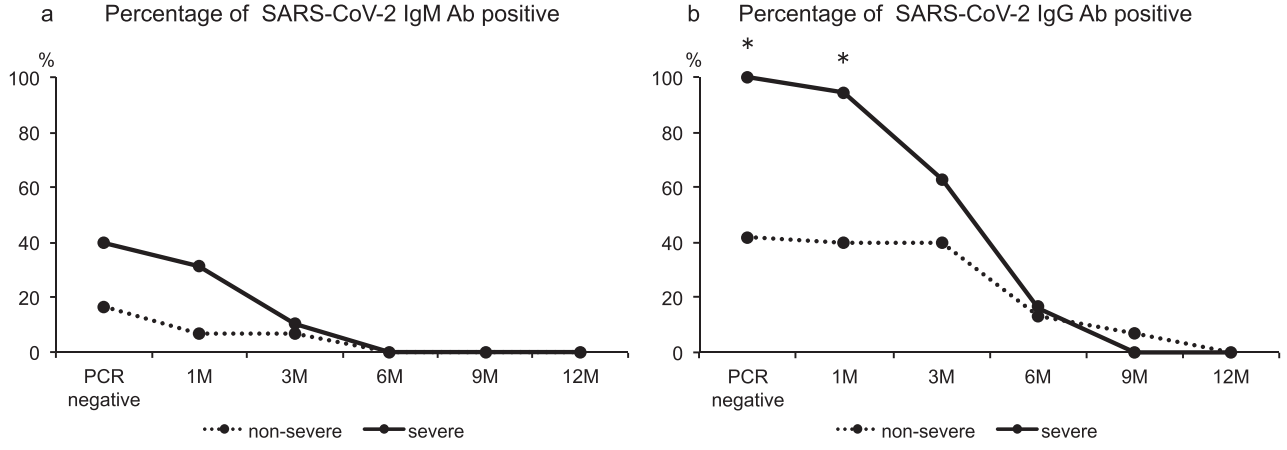

Fig. 2. Rate of positivity for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) IgM and IgG antibodies at the point of testing negative for SARS-CoV-2 by polymerase chain reaction and one, three, nine, and twelve months later for patients in the non-severe (n=15) and severe (n=19) groups. $^{*}P<0.05$.

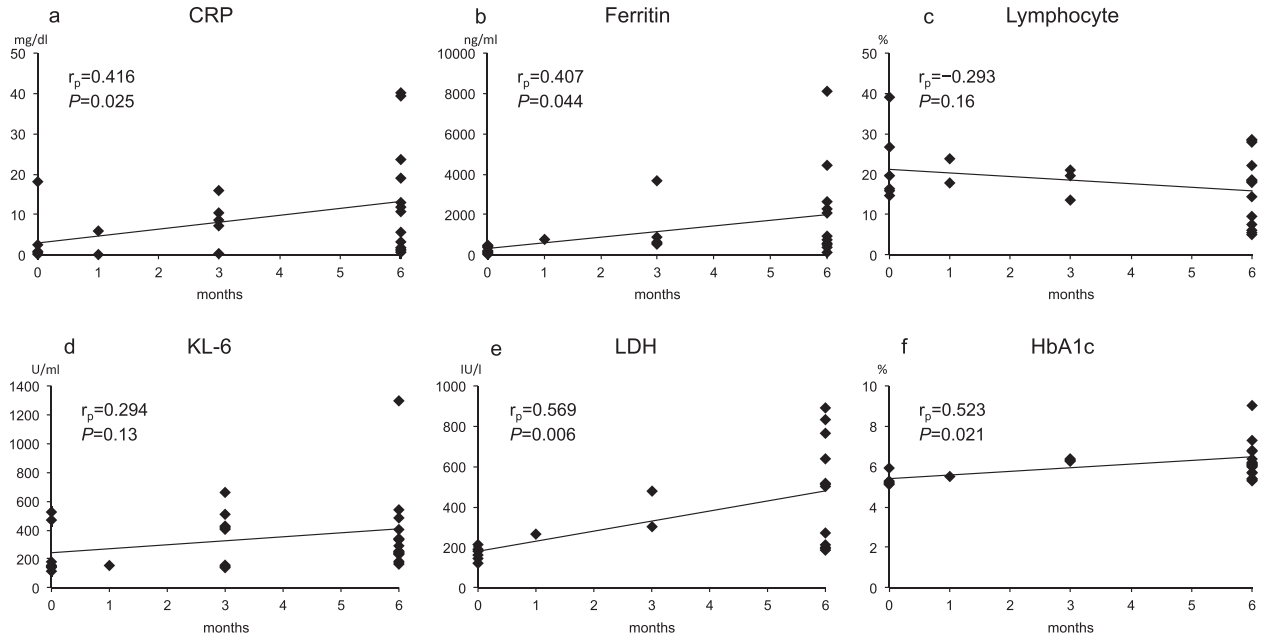

Fig. 3. Pearson correlation between the biomarkers from the blood examination at admission and the negative testing date for severe acute respiratory syndrome coronavirus 2 IgG antibodies. $^*P<0.05$ (n=34).

tested negative for IgG antibodies; most patients may test negative for both IgM and IgG antibodies after six-nine months. Zhao *et al*. investigated the expression of SARS-CoV-2 IgM and IgG antibodies in 173 patients with COVID-19[29]. The positivity rates for IgM and IgG antibodies on Day 15 after the onset of COVID-19 were 94.3% and 79.8%, respectively. In the present study, the positivity rate for SARS-CoV-2 IgM antibodies was lower than that for IgG antibodies. One possible reason for the discrepancy between our data and the other data is that the measurement kits used in each study and their performance differed.

In our study, the positivity rate for SARS-CoV-2 IgG antibodies at the time of PCR negativity was 41.7% for the non-severe group and 100.0% for the severe group, which may suggest that IgG antibodies are more likely to be produced in patients with severe disease. In this study, the positivity rate for IgG antibodies declined over time, regardless of severity. There was no difference in the percentage of patients with non-severe and severe diseases after three months. Additionally, the antibody was almost absent six to nine months after the date of PCR negativity.

The major structural proteins of SARS-CoV-2 include envelope, membrane, spike, and nucleocapsid proteins, and antibodies are thought to be produced mainly against the spike and nucleocapsid proteins[30]. Premkumar *et al*. reported that SARS-CoV-2 IgG antibodies target the S-binding domain, suggesting they may be able to neutralize SARS-CoV-2[26]. Our investigation showed that most patients with severe COVID-19 are more likely to produce SARS-CoV-2 IgG antibodies, which are assumed to be neutralizing antibodies, leading to the prevention of SARS-CoV-2 reinfection. However, more than 80% of patients with COVID-19 lose the ability to produce IgG antibodies within six months after testing negative for SARS-CoV-2 by PCR, regardless of the severity, indicating that most patients with COVID-19 may not effectively neutralize infection at that time.

Additionally, we found that the concentrations of CRP, ferritin, LDH, and HbA1c in the blood were correlated with the time of testing negative for SARS-CoV-2 IgG antibodies. Further, we showed that these markers were significantly higher in patients with severe disease than those without the severe disease. Furthermore, the rate of IgG antibody positivity was higher in patients with severe disease than in those without severe disease after three months of testing negative for SARS-CoV-2 by PCR. These results suggest that differences may influence the correlations between these biomarkers in the production of IgG antibodies depending on the severity of COVID-19.

This study has several limitations. First, the participants were in patients, and the number of asymptomatic or mild COVID-19 patients was relatively low. Second, SARS-CoV-2 IgM and IgG

antibodies were measured from the time of testing negative for SARS-CoV-2 by PCR, and the antibody production status from the onset of the disease to that time has not been investigated. Therefore, whether patients with COVID-19 began antibody production at disease onset was not established.

Conclusions

The patients with severe COVID-19 had a higher positivity rate for SARS-CoV-2 IgG antibodies, which are considered neutralizing antibodies, at the time of COVID-19 improvement. Six months after testing negative for SARS-CoV-2 by PCR, most patients tested negative for IgG antibodies regardless of severity. These results suggest that in patients with COVID-19, neutralizing antibodies are more likely to be detected in severe cases of COVID-19; however, most patients lose the ability to produce neutralizing antibodies against SARS-CoV-2 within six months, regardless of severity. Therefore, it is essential to maintain proper infection control measures even after being infected with SARS-CoV-2.

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Conflicts of interest disclosure

No potential conflict of interest was disclosed.

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