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Association between SARS-CoV-2 anti-spike antibody titers and the development of post-COVID conditions: A retrospective observational study

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Abstract: The symptoms that persist after an acute coronavirus disease 2019 (COVID-19) are referred to as post-COVID conditions. Although the cause of post-COVID conditions remains unclear, the host immune response to SARS-CoV-2 may be involved. Hence, we aimed to investigate the effect of serum antibody titers against SARS-CoV-2 on the development of post-COVID conditions. We conducted a retrospective observational study of COVID-19-recovered individuals who attended the clinic at the National Center for Global Health and Medicine between January 2020 and April 2021. Serum SARS-CoV-2 anti-spike antibody titers were measured and a questionnaire survey was used to collect information on the presence of post-COVID conditions and demographic characteristics of the participants. Participants were then divided into two groups: high peak antibody titer group [≥ 0.759 OD450 value], and low peak antibody titer group [< 0.759 OD450 value] and compared their frequency of post-COVID conditions. Of 526 individuals attending the clinic, 457 (86.9%) responded to the questionnaire. We analyzed the data of 227 (49.7%) participants with measurements of serum antibody titers during the peak period. The incidence of depressed mood was significantly higher in the group with higher antibody titers (odds ratio: 2.34, 95% CI: 1.17–4.67, $p = 0.016$). There was no significant difference in the frequency of the remaining symptoms between the two groups. Among post-COVID conditions, the depressed mood was more frequent in the group with high serum antibody titers which suggests a difference in pathogenesis between depressive mood and other post-COVID conditions that requires further investigation.

***Keywords*:** antibody titer, COVID-19, post-COVID condition, questionnaire survey, SARS-CoV-2

Introduction

Coronavirus disease 2019 (COVID-19) is a global threat and has caused many deaths; furthermore, the sequelae of COVID-19, known as post-COVID conditions, also have a considerable social impact.

Several studies have identified risk factors for the development and progression of post-COVID conditions (*1-4*). However, the pathophysiology of these conditions remains unclear. The possible pathogenesis of post-COVID conditions has been classified by the National Institute for Health Research according to at least four categories (*5-6*), and inadequate antibody response is one of the potential underlying mechanisms that has been identified.

It has been reported that vaccination is effective in the prevention and treatment of post-COVID conditions and multisystem inflammatory syndrome in children (*7-9*), suggesting that the host antibody response may be involved in the development of these conditions. This study investigated the relationship between the development of post-COVID conditions and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) anti-spike serum antibody titers.

Materials and Methods

Questionnaire

We conducted a retrospective observational study at an outpatient clinic of the Disease Control and Prevention Center in the National Center for Global Health and

Medicine (NCGM), Tokyo, Japan. In February 2022, we mailed a self-report paper-based questionnaire on post-COVID conditions to individuals aged 20 years or over who had recovered from acute COVID-19 and attended the outpatient clinic in NCGM for a pre-donation screening test for COVID-19 convalescent plasmapheresis (*10*) between January 2020 and April 2021. Patients underwent testing for SARS-CoV-2 anti-spike serum antibody titers at the visit. The questionnaire also included the demographic characteristics of the participants.

Participants were asked to complete and return the questionnaire. Participation was voluntary and confidential and reminders were sent to patients at 2 weeks and 1 month after mailing the questionnaire. The questionnaire was based on questionnaires from previous studies and discussions among the authors (*1,2,11-14*). The questionnaire content is described in our previous study (*4*). The following post-COVID conditions were assessed in the questionnaire: fatigue, cough, dysosmia, dysgeusia, shortness of breath, hair loss, depressed mood, loss of concentration, and memory disturbance. These were classified as ongoing or late-onset symptoms, as described in our previous study (*4*). Furthermore, the severity of COVID-19 was categorized according to previously published reports (*1,2*): *i*) mild, no oxygen therapy; *ii*) moderate, oxygen therapy without mechanical ventilation; *iii*) severe, mechanical ventilation with or without extracorporeal membrane oxygenation. Since the most of participants were treated at other medical facilities, we were unable to verify the severity of illness in their medical records and collected information only from the results of the questionnaire.

Measurement of SARS-CoV-2 anti-spike antibody titers

Recombinant SARS-CoV-2 spike protein (full-length) was purified using Expi293 expression system and coated on the MaxiSoap 96 well enzyme-linked immune-sorbent assay plate (ThermoFisher Scientific, Waltham, MA) overnight at 4ºC. After blocking with 1% BlockAce (KAC, Kyoto, Japan), the 1/100 diluted patient serum samples were applied, and then incubated with anti-human IgG conjugated with horseradish peroxidase (GeneTex, Irvine, CA). The captured anti-spike antibody titers were detected with 3,3',5,5'-tetramethylbenzidine substrate solution (Nacalai Tesque, Kyoto, Japan) and their absorbance (OD450) was measured at 450 nm wave-length using a microplate reader (Bio-Rad, Irvine, CA). The healthy volunteer serums without SARS-CoV-2 infection were used as negative control, whereas the infected patients' serums with high amount of anti-spike antibodies were used as positive control. Each sample was assayed in triplicates.

SARS-CoV-2 anti-spike antibody titers

SARS-CoV-2 anti-spike antibody titers vary according to the time between the onset of acute COVID-19 and testing. Sera were collected between 21 and 60 days after COVID-19 onset, based on previous literature (*15-18*). COVID-19 onset was defined as the date of first appearance of any symptoms associated with acute COVID-19 or the date of diagnosis of COVID-19 in asymptomatic patients. Patients were divided into two groups according to the levels of serum antibody titers, as follows: [≥ 0.759 OD450 value] (high peak antibody titer group) and [< 0.759 OD450 value] (low peak antibody titer group). This is because the cut-off value of antibody titers was not defined and the median antibody titer for this study participants was 0.759 OD450 value.

Statistical analysis

We compared the frequency of each post-COVID condition collected by the questionnaire between two groups, classified according to the SARS-CoV-2 anti-spike antibody titers, using Chi-square tests (or Fisher's exact test if the expected frequency was < 5). To adjust for potential confounders, observed differences in baseline characteristics (sex, age, obesity [body mass index > 25 kg/m^2], smoking, hypertension, diabetes, dyslipidemia, bronchial asthma, severity of acute COVID-19, and administration of antiviral medications and steroids) between the two groups were controlled for by using an inverse probability weighting (IPW)-adjusted analysis (*3,4*). The stabilized weight of each case was based on the propensity score which was calculated by a multivariate logistic regression model predicting the likelihood of having higher/lower SARS-CoV-2 anti-spike antibody titer. Hypertension, dyslipidemia, diabetes, and bronchial asthma were chosen as the confounding variables because of their high prevalence among the participants. Whereas, malignancy and chronic obstructive pulmonary disease, which are also risk factors for severe COVID-19, were not included as variables for adjustment because of their relatively low prevalence. The balance in covariates between the two groups before and after IPW adjustment was assessed using the standardized mean difference (SMD) and a difference in SMD above 20% was interpreted as a meaningful imbalance (*19*). After the IPW adjustment, we performed a generalized linear model analysis to estimate the average treatment effects of higher SARS-CoV-2 anti-spike antibody titers on the development of post-COVID conditions.

P values < 0.05 were considered statistically significant. Sensitivity analyses were performed using two other cut-off values of COVID-19 antibody titers (0.6 and 0.9 OD450 value). Stata 17.0 (StataCorp, College Station, TX, USA) was used to perform all analyses.

Sensitivity analyses

We performed sensitivity analyses with different cut-off values of SARS-CoV-2 anti-spike antibody titers (0.6 and 0.9 OD450 value) because the cut-off value of antibody titers was not defined.

Ethics approval and informed consent

The study conformed to the provisions of the Declaration of Helsinki (as revised in 2013) and the study protocol was approved by the Ethics Committee of the Center Hospital of the NCGM (NCGM-G-004121-00). All study participants provided written informed consent before answering the questionnaire.

Results

Participant characteristics

Of the 526 potentially eligible participants, 457 (86.9%) answered the questionnaire. Of these, 227 (49.7%) had antibodies measured 21–60 days after the onset of COVID-19 and were eligible for inclusion in the analysis. We excluded 230 patients because their serum antibody titers were not measured during the peak period. The characteristics of the participants analyzed are shown in Table 1. The median age was 47 years, 101 participants (44.5%) were male, and 117 (51.5%) had no underlying disease. All of the participants were Japanese. Seventy-one participants (31.3%) had experienced COVID-19 pneumonia. The severity of COVID-19 was mild in 193 (85.0%), moderate in 27 (11.9%), and unknown in the remaining 7 participants. No participants had experienced severe disease.

SARS-CoV-2 anti-spike antibody titers

SARS-CoV-2 anti-spike antibody titers among the study participants did not follow a normal distribution (Figure 1). The median antibody titer was 0.759 [IQR: 0.311–1.348] OD450 value; therefore, analysis was performed between two groups: those with antibodies < 0.759 (*n* =

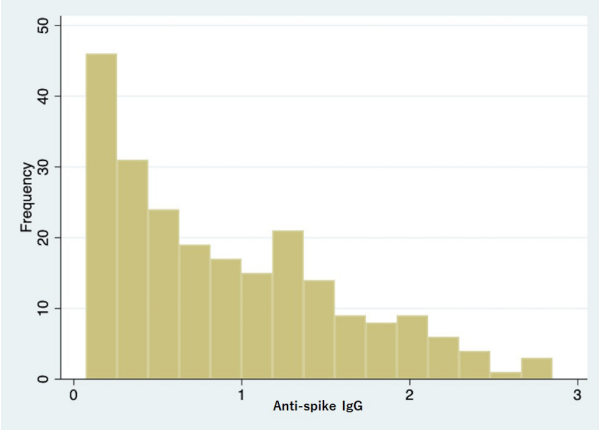

Figure 1. Distribution of SARS-CoV-2 anti-spike antibody titers among the study participants.

Table 1. Characteristics of the study participants

Characteristic	Overall (*n* = 227)	Low antibody titer (*n* = 114)	High antibody titer (*n* = 113)	SMD (before IPW)	SMD (after IPW)	Missing
Age (years), median [IQR]	47 [40–54]	43 [37–51]	51 [45–57]	0.731		0.070
Male sex	101	40	61	0.395	0.126	
Obesity (BMI > 25 kg/m^2)	80	34	46	0.181	0.112	
Smoking	73	38	35	0.024	0.014	
No underlying disease	117	60	57			
Hypertension	35	7	28	0.596	0.020	
Diabetes	17	6	11	0.160	0.057	
Dyslipidemia	30	11	19	0.198	0.006	
Asthma	33	15	18	0.061	0.041	
COPD	1	1	0			
Malignancy	4	1	3			
Use of antivirals	40	6	34	0.382	0.098	13
Use of steroids	26	6	20	0.135	0.164	23
Mild severity	193	90	103			
Moderate severity	27	4	23	0.531	0.098	7
Fatigue	150	73	77			1
SoB	81	37	44			1
Cough	110	50	60			
Dysosmia	118	74	44			
Dysgeusia	97	55	42			
Hair loss	50	21	29			2
Depressed mood	66	28	38			1
LoC	70	30	40			
MD	46	19	27			3

Abbreviations: BMI, body mass index; COPD, chronic obstructive pulmonary disease; IPW, inverse probability weighting; IQR, interquartile range; LoC, loss of concentration; MD, memory disturbance; SMD, standardized mean difference; SoB, shortness of breath.

Table 2. Association between SARS-CoV-2 anti-spike antibody titers and development of post-COVID conditions

Characteristic	Odds Ratio	95% Confidence Interval	*p* value
Fatigue	1.55	0.72–3.31	0.261
Cough	1.88	0.97–3.66	0.061
Dysosmia	0.61	0.30–1.23	0.167
Dysgeusia	1	0.51–1.94	> 0.99
SoB	1.34	0.65–2.77	0.419
Hair loss	1.92	0.91–4.09	0.089
Depressed mood	2.34	1.17–4.67	0.016
LoC	1.85	0.93–3.67	0.079
MD	2.11	0.98–4.52	0.056

Abbreviations: LoC, loss of concentration; MD, memory disturbance; SoB, shortness of breath. Odds ratio indicates the incidence rate ratio of each symptom in the group with higher SARS-CoV-2 anti-spike antibody titers compared to the group with lower antibody titers.

114) and those with antibodies ≥ 0.759 (n = 113).

Correlation between SARS-CoV-2 anti-spike antibody titers and development of post-COVID conditions

The frequencies of each post-COVID condition between the two groups with the high and low antibody titers were analyzed after adjusting for potential confounding factors. There was no significant difference between the two groups in the incidence of fatigue, cough, dysosmia, dysgeusia, shortness of breath, hair loss, loss of concentration, and memory disturbance (Table 2). In contrast, the incidence of depressed mood was significantly higher in the group with a higher antibody titer (OR: 2.34, 95% CI: 1.17–4.67, p = 0.016).

Sensitivity analyses

The same analyses were performed using the antibody titer cut-off values of 0.6 and 0.9 as sensitivity analyses. The antibody titer cut-off value was set at 0.6 and the frequencies of post-COVID conditions were analyzed in the two groups: patients with antibodies < 0.6 (n = 99) and patients with antibodies ≥ 0.6 (n = 128). No significant difference was observed between the two groups in the incidence of fatigue, cough, dysosmia, dysgeusia, shortness of breath, hair loss, and loss of concentration. The incidence of depressed mood (OR: 2.70, 95% CI: 1.35–5.38, p = 0.005,) and memory disturbance (OR: 2.42, 95% CI: 1.14–5.16, p = 0.021) was significantly higher in the group with higher antibody titer (Online Data Tables S1 and S2, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=65*).

The antibody titer cut-off value was set at 0.9 and the frequencies of post-COVID conditions were analyzed in the two groups: patients with antibodies < 0.9 (n = 127) and patients with antibodies ≥ 0.9 (n = 100). No significant difference was observed between the two groups in the incidence of all the symptoms, but the incidence of depressed mood tended to be higher in the group with a higher antibody titer (OR: 1.85, 95% CI: 0.93–3.68, p = 0.081) (Online Data Tables S3 and S4, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=65*). The results of the sensitivity analyses showed that changing the cut-off value of the antibody titer did not substantially affect the difference in frequencies of post-COVID conditions between the two groups.

Discussion

In this study, we investigated the effect of antibody titers on the development of post-COVID conditions. We analyzed only participants whose antibodies were tested at a time when antibody titers were likely to be elevated. There was no significant difference in the frequency of ongoing and late-onset symptoms other than depressed mood according to the SARS-CoV-2 anti-spike antibody titers.

A literature search did not reveal any studies showing an association between the SARS-CoV-2 anti-spike antibody titers and the development of post-COVID conditions. A study comparing recovering COVID-19 patients who developed post-COVID conditions with those who did not, found no significant difference between the groups in the magnitude of the antibody titers (*20*), which is consistent with the results of the present study. A previous study suggested that post-COVID-19 conditions are caused by a combination of four conditions: persistent viral infection, reinfection, inadequate immune response, and myalgic encephalomyelitis/chronic fatigue syndrome (*5*). The lack of association between the development of post-COVID conditions and antibody titers can be partly attributed to the combination of multiple mechanisms (*21*).

Depressed mood was the only symptom that was associated with elevated SARS-CoV-2 anti-spike antibody titers in this study. The mechanism by which depressed mood occurs after COVID-19 recovery is not clearly understood, although it has been reported that longer periods of isolation due to COVID-19 might be associated with an increased risk of having depression and anxiety (*22*). Antibody titers are considered to reflect the level of the immune response to COVID-19, and higher levels are more likely to be found in patients with a strong immune response and prolonged period of isolation in the hospital, which may explain the increased risk of developing a depressed mood. In addition, Song *et al.* reported that intrathecal SARS-CoV-2 antibody was associated with neurological symptoms caused by COVID-19 (*23*). Further investigation of the association between the SARS-CoV-2 antibody titers in both cerebrospinal fluid and blood, and neurological post-COVID conditions, including depressed mood, will lead to a more detailed understanding of the pathogenesis of

the diseases.

This study has several limitations. First, the SARS-CoV-2 anti-spike antibody titers were measured only at a time when they were likely to be elevated, so it is unclear whether persistence of high antibody titers is associated with the development or persistence of post-COVID conditions. Moreover, it is possible that participants with low antibody titers at the time of measurement yet with elevated antibody titers at other times were included in the study. Second, only the symptoms extracted from the questionnaire were analyzed in this study; hence, not all post-COVID conditions were investigated. Third, this study was based on a self-reported questionnaire-based survey, which was subject to various biases, such as selection, volunteer, and recall biases. Fourth, the frequency of post-COVID conditions and their association with antibody titers may be altered in epidemic strains that differ from those at the time of the study. Finally, the association between vaccination and the development of post-COVID conditions is unclear because the vaccination history of participants was not obtained in this study. However, considering the timing of the antibody titer measurements, it is likely that few vaccinated participants were included and we reasonably consider that this study evaluated the SARS-CoV-2 anti-spike antibody titers in unvaccinated persons.

In conclusion, the association between the development of specific post-COVID conditions symptoms and antibody titers was investigated, and no association was found except for that between high antibody titers and depressed mood. We postulate that the difficulty in detecting an association between the development of post-COVID conditions and antibody titers is because these conditions are likely to develop through multiple mechanisms. This study suggests that there is a difference in the underlying pathogenic mechanisms between depressive mood and other post-COVID conditions, and further research is needed to investigate this.

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