Analysis of Serum Antibody Level after COVID-19 Vaccine and Side Effects after Vaccine

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Abstract

Background: COVID-19 has caused a large number of deaths, in the elderly. In this situation, vaccines have been considered the main strategy to combat COVID-19 and were developed worldwide in 2020. One problem with COVID-19 vaccination is side effects after vaccination. In this report, we identify the relationship between side effects and elevation of antibodies as determined by monitoring several kinds of antibodies against COVID-19. **Objective:** We investigated whether the antibody level changed depending on side effects after COVID-19 vaccination. **Methods:** Healthy volunteers, who received two vaccinations between 10 March and 7 May 2021, were collected for this study. Information including age, gender, smoking history, medical history including allergies and side reactions after vaccination was obtained by questionnaire. Serum levels of antibodies of IgG and IgM against each S1, SP, and NP antigens of COVID-19 were evaluated frequently for 3 - 4 months after the first vaccination. **Result:** Ten employees working at Iwate Medical University were evaluated in this study. Side effects were observed in 7 of 10 patients, and grade 2 side effects in all 3 patients with a history of allergic disease. Serum S1 and SP IgG were elevated sufficiently in all patients. In all patients, IgG antibody titers fell below the cutoff point in approximately 3 months. No cases had elevated NP antibodies. SP IgM was elevated in three cases; all three cases with elevated IgM had allergic disease and the degree of side effects was relatively higher. Subjects with long-lasting elevated SPIgM were observed. **Conclusion:** S1 IgG and SP IgG exceeded the cutoff in all subjects after vaccination but decreased below the cutoff in all subjects within 4 months, regardless of side effects or allergic history. On the other hand, elevated SPIgM was suspected to be related to side effects and history of allergies, and cases with persistent elevation of SPIgM were observed.

1. Introduction

COVID-19 has caused a large number of deaths, in the elderly. In this situation, vaccines have been considered the main strategy to combat COVID-19 and were developed worldwide in 2020. This new coronavirus attaches to cells with spike proteins that protrude from the surface. The spike proteins are composed of two functional domains: S1, containing a receptor binding domain (RBD), and S2, containing a membrane fusion peptide (FP) [1]. The receptor of the virus is ACE2 (angiotensin converting enzyme 2), bound to the S1 region of the virus. Antibodies targeting S1 (RBD) can be expected to have a neutralizing function [2] [3].

We have been using one of RNA vaccines, originally known as the Pfizer-BioNTech COVID-19 Vaccine, and now to be marketed as Comirnaty®, for the prevention of COVID-19 disease. The vaccine is composed of RNA encoding the spike protein of the SARS-COV-2 virus and lipids. An mRNA vaccine has not been developed in humans so far, and Comirnaty® was the first to be approved in the US. An RNA vaccine can be safely developed using only the protein information of the target virus, without the need to culture a virus showing pathogenicity. This was expected to shorten the development time. In fact, the vaccine was approved in the United Kingdom on December 3, 2020, and became the earliest clinically available COVID-19 vaccine in the world. In Japan, it was approved on February 12, 2021, and inoculation for medical staff began soon after.

The effectiveness of vaccination needs to be assessed. The principal evaluation method is to compare COVID-19 prevalence in vaccinated and unvaccinated individuals. However, this was not deemed an appropriate method for this study as it would require a large number of people due to the low infection rate in this part of Japan. Therefore, we focused on the antibody titer against COVID-19. After vaccination, we evaluated the titer of COVID-19 antibodies at regular intervals. This provided information on the peak titer and duration of antibody titer elevation, allowing the effectiveness of the COVID-19 vaccine to be assessed from one aspect.

One problem with COVID-19 vaccination is the side effects after vaccination. There are a variety of side effects, the main symptoms being fever, fatigue, systemic myalgia, and so on. Some people have to be absent from work or school after vaccination due to severe side effects. The association between the severity of side effects and the titer of serum antibodies representing immune activity against COVID-19 has not been elucidated sufficiently. In this report, we identify the relationship between side effects and elevation of antibodies as determined

by monitoring several kinds of antibodies against COVID-19.

2. Methods

2.1. Subjects

We recruited subjects who cooperated with blood tests in the hospital. We enrolled staff working at Iwate Medical University who received two vaccinations between March 10 and May 7, 2021 and obtained their consent. The only requirement for participation was having two doses of the vaccine. Subjects included physicians, clerks, and research assistants. Enrolled persons underwent blood examinations at several time points as described below. Participants were to receive a total of 11 blood tests. Participants who could not receive more than 5 tests were excluded from the study. In this study, persons previously infected with COVID-19 were excluded.

2.2. Questionnaire

The questionnaire (S1) was prepared by the research secretariat. The questionnaire asked the following questions. Their age, gender, smoking history, medical history, allergies. Date of vaccination. Post-vaccination body temperature and adverse events and medications used for adverse events were investigated.

2.3. Vaccination

Pfizer's coronavirus-modified uridine RNA vaccine (SARS-CoV-2), Comirnaty®, was used. The vaccines were inoculated into the central part of the deltoid muscle according to the manual. All subjects had a second vaccination about 3 weeks later.

2.4. COVID-19 Antibodies

We analyzed IgG and IgM antibodies against S1, SP, and NP. Both S1 and SP work against part of the spike protein. The spike protein of SARS-CoV-2, which plays a key role in the receptor recognition and cell membrane fusion process, is composed of two subunits, S1 and S2. The S1 subunit contains a receptor-binding domain that recognizes and binds to the host receptor angiotensin-converting enzyme 2 (ACE2), while the S2 subunit mediates viral cell membrane fusion by forming a six-helical bundle via the two-heptad repeat domain. SP is considered as S1 and S2. The S1 antibody binds to the S1 part, and the binding part of the SP antibody is considered to straddle the S1 part and the S2 part. Nucleocapsid (NP) is expressed at higher levels in infected cells and is highly immunogenic, but the Pfizer-BioNTech COVID-19 vaccine has no binding site of NP. In this study, elevated NP antibody was considered a marker of previous COVID-19 infection.

2.5. Antibody Tests

A venous blood sample was drawn from the vein into a 5 - 7 mL vacuum tube

(Venoject II VP-P050K, TERUMO Corp., Tokyo, Japan). After blood collection, the blood sample was allowed to stand for 15 minutes. Centrifugation (1200 G × 10 min) was performed within 4 hours. After centrifugation, the sample was stored at −30˚C. For analysis, QuaResearch COVID-19 Human IgM IgG ELISA Kit (Nucleocapsid Protein) (RCOEL961N; Cellspect Co., Ltd.), QuaResearch COVID-19 Human IgM IgG ELISA Kit (Spik Protein-S1) (RCOEL961 S1; Cellspect Co., Ltd.) and QuaResearch COVID-19 Human IgM IgG ELISA kit (Spike Protein-S1 + S2) (RCOEL961 SP; Cellspect Co., Ltd) were used in Cellspect. QuaResearch COVID-19 Human IgM IgG ELISA Kit (Nucleocapsid Protein) (RCOEL961N; Cellspect Co., Ltd.) was used to measure anti-Nucleocapsid Protein (NP) IgM and NP IgG. Anti-Spike protein-S1 (S1) IgM and S1 IgG were measured using the QuaResearch COVID-19 Human IgM IgG ELISA Kit (Spike Protein-S1) (RCOEL961S1; Cellspect Co., Ltd.). Anti-Spike protein-S1 + S2 (SP) IgM and SP IgG were measured using the QuaResearch COVID-19 Human IgM IgG ELISA Kit (Spike Protein-S1 + S2) (RCOEL961S1; Cellspect Co., Ltd.). Serum samples were diluted 1:1000 in 1% bovine serum albumin/phosphate buffered saline with Tween-20 (PBST) for ELISAs with N, S1 or S1 + S2 proteins. After incubation at room temperature for 1 hour, specific antibodies were detected with horseradish peroxidase-conjugated antihuman IgM or IgG at room temperature for 1 hour. The enzymatic reaction was developed with TMB (3,3',5,5'-tetramethylbenzidine) substrate at room temperature for 10 min. The reaction was stopped using 1M HCl, and the plates were read at 450 nm with plate reader SH-1000 (CORONA Electric Co., Ltd.) in accordance with the manufacturer's measurement protocol. According to the manufacturer's protocol, cutoffs of infected persons are 0.14, 0.5, 0.3, 0.11, and 0.3 for SP IgG, NP IgG, S1 IgG, SP IgM, and NP IgM, respectively. Since the cutoff value of S1 IgM has not been determined, the test for S1 IgM was excluded from this study.

Participants were tested for antibodies containing S1 IgG, SP IgG, NP IgG, SP IgM, and NP IgM once before vaccination and seven times at weekly intervals after the first vaccination. Thereafter, participants underwent a blood antibody test once a month for 4 months.

2.6. Evaluation of Adverse Events

Using a daily health check sheet, we confirmed the symptoms after vaccination. Symptoms were evaluated based on the CTCAE (Common Terminology Criteria for Adverse Events) v 5.0. Pain, redness, and swelling at the injection site, all local reactions, were distinguished from systemic symptoms. Headache, arthralgia, myalgia, chills, fever, and malaise occurring within 24 hours after vaccination were evaluated as systemic side reactions. Grade refers to the severity of the Adverse Events. Grade 1 is mild symptoms. Grade 2 has moderate symptoms and requires local therapeutic intervention.

2.7. Statistics

We compared the elevation peak of antibodies to side effects and allergic history

with the Mann-Whitney U test. This was performed with Easy R (EZR, Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). More precisely, it is a modified version of R Commander designed to add statistical functions frequently used in biostatistics.

3. Results

Consent was obtained from 16 of the health care workers who received the vaccine twice between March 10 and May 7, 2021. We analyzed 10 subjects, excluding 5 subjects who could not perform sufficient blood tests and 1 subject who was infected with the new coronavirus (Figure 1). The subjects were 8 men and 2 women, aged between 29 and 56 years, with a median of 40 years. There were 9 non-smokers and 1 smoker. Three patients have allergic history including 2 bronchial asthma and 1 atopic dermatitis. (Table 1).

After the first vaccination, 2 subjects had grade 1 side effects and 2 subjects had grade 2 side effects. After the second vaccination, 2 subjects had grade 1 side effects and 4 subjects had grade 2 side effects. Three people did not develop any side effects through the first and second rounds (Table 2(a)).

The most common side effect after the first and the second vaccination was malaise. Headache and fever were the second most common side effects. Both the frequency and grade of side effects were greater after the second vaccination than the first.

All side effects appeared within 3 days after vaccination (Table 2(b)). Side effects were observed in 7 out of 10 patients, and all 3 patients with a history of allergic diseases, bronchial asthma and atopic dermatitis had grade 2 side effects (Table 2(c)).

Comparing the peak values of each antibody, both SP IgG and S1 IgG exceeded the cutoff values in all cases. No cases showed elevated NP antibodies because the vaccine does not produce nucleocapsid protein.

Elevated SPIgM was observed in three cases. (Figure 2). The three cases with significant elevations all had allergic diseases and higher degree of side effects.

The antibody levels of both SP IgG and S1 IgG were below the cutoff value
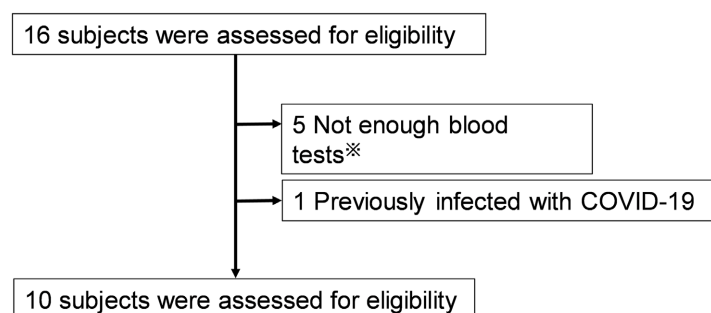

Figure 1. Consort diagram. This study excluded infected individuals. * 5 participants were excluded due to an insufficient number of blood tests. The number of required antibody tests was defined as 5 or more.

Table 1. Subject characteristics.

	All (N = 10)
Age	
Median (range)	40 (29 - 56)
Sex	
Male	8
Female	2
Smoking status	
Current or former	1
Never	9
Medical history*	
Asthma	2
Atopic dermatitis	1
Hyperlipidaemia	1
Hypertension	1
Sinusitis1	
None	5

*One subject has sinusitis and asthma.

Table 2. Adverse events that occurred 6 hours after vaccination (Excludes injection site reactions).

(a)

Adverse events	after the first vaccination	after the second vaccination
Only Grade 1	2	3
Grade 2	2	4
None	6	3

(Number of people)

(b)

Adverse events	after the first vaccination		after the second vaccination	
	Grade1	Grade2	Grade1	Grade2
Malaise	2	1	4	3
Headache	1	0	4	1
Fever	0	0	2	2
Chills	0	0	3	0
Arthralgia	0	2	0	1
Myalgia	0	0	1	0

(One subject has multiple adverse events)

All adverse events appeared within 3 days after vaccination.

(c)

		History of allergic disease	Adverse events	
			Only Grade 1	Grade 2
56	M		—	—
46	M	+		+
46	F		—	—
42	M		—	—
41	M	+		+
39	M			+
37	M		+	
32	M		+	
32	M			+
29	F	+		+

before vaccination in all cases but exceeded the cutoff value in all cases within 20 to 40 days after the first vaccination. The peak was observed within 20 to 80 days. Most subjects' antibody titers on blood tests peaked after the second vaccination. Of the 9 cases that were followed for 120 days after the first vaccination, only 1 case was below the cutoff value for SPIgG, but 6 cases were below the cutoff value for S1 IgG (Figure 3(a), Figure 3(b)).

The antibody level of SP IgM was below the cutoff value in all cases before vaccination but exceeded the cutoff value in 9 patients after the first vaccination. One patient exceeded the cutoff value for the first time 140 days after vaccination. There was one subject who never exceeded the cutoff value during the follow-up period. The peak was observed within 20 to 60 days. At the time of final confirmation, 120 to 160 days after vaccination, the antibody titer of 5 patients continued to exceed the cutoff value (Figure 3(c)).

When the SP IgG values were compared with and without the side effects of G2, the median of 5 cases that had the side effects of G2 was 0.447, and the median of 5 cases that did not have the side effects of G2 or higher was 0.347. In the comparison of the S1IgG values, the median of 5 patients who had side effects of G2 was 1.059, and the median of 5 patients who did not have side effects of G2 was 1.077. Comparing the SP IgM values, the median of 5 patients who had side effects of G2 was 0.126, and the median of 5 patients who did not have side effects of G2 was 0.102 (Figure 4(a)).

Comparing the SP IgG values with and without allergic disease, the median of 3 people with a history of allergic disease was 0.447, and the median of 7 people without a history of allergic disease was 0.378.

A comparison of S1 IgG levels also showed that the median of 3 people with a history of allergic disease was 0.732 and the median of 7 people without a history of allergic disease was 1.107. On the other hand, a comparison of SP IgM values
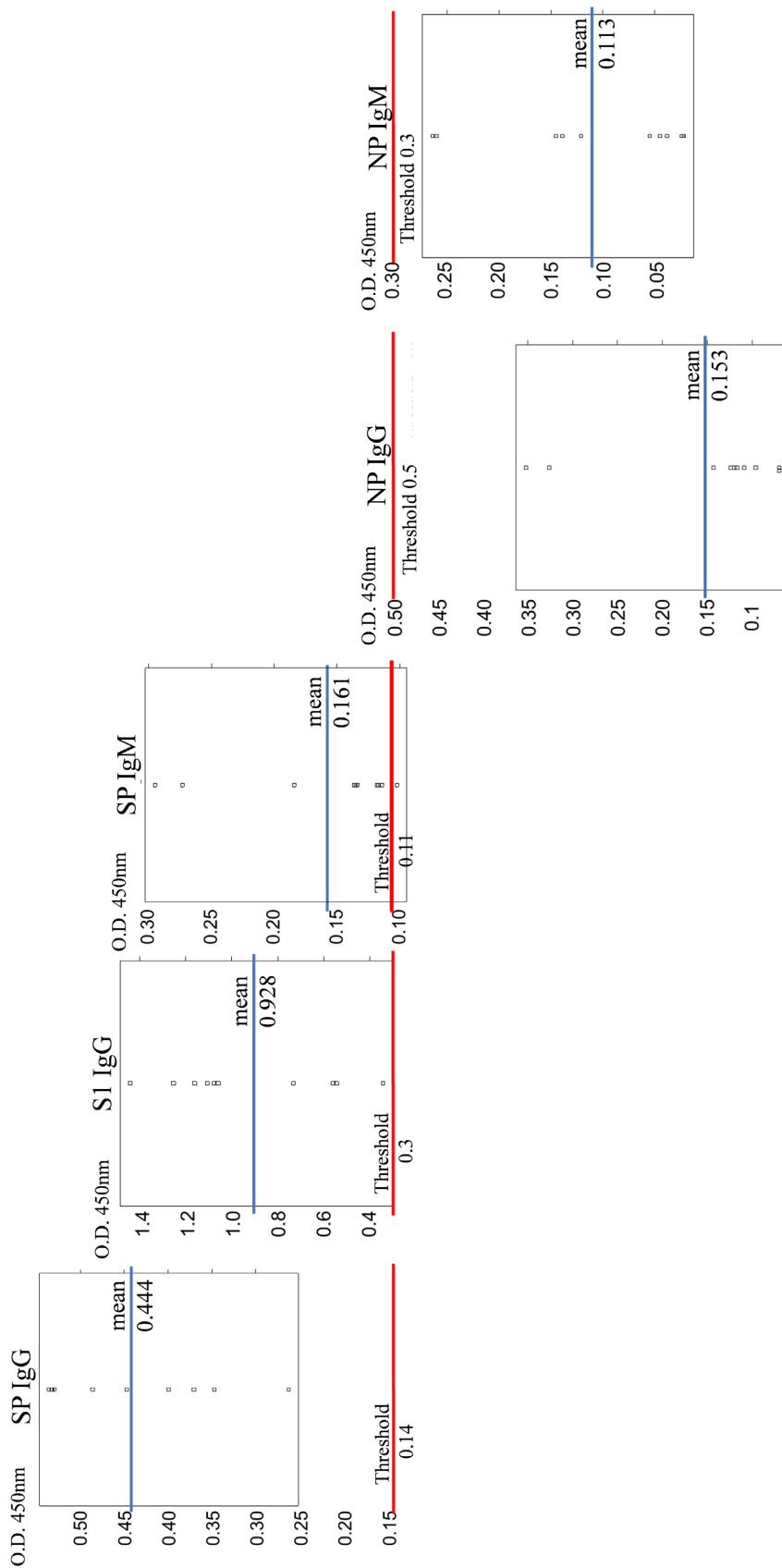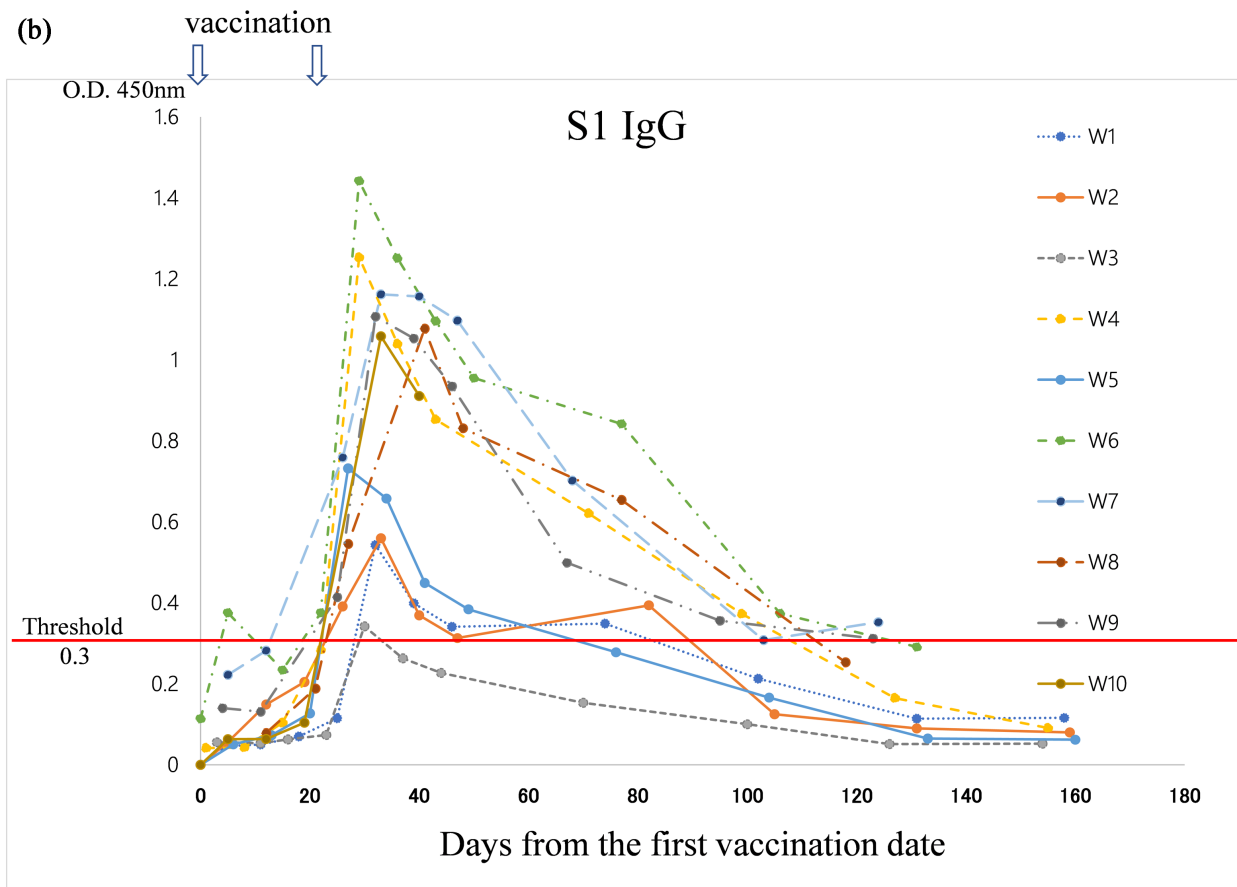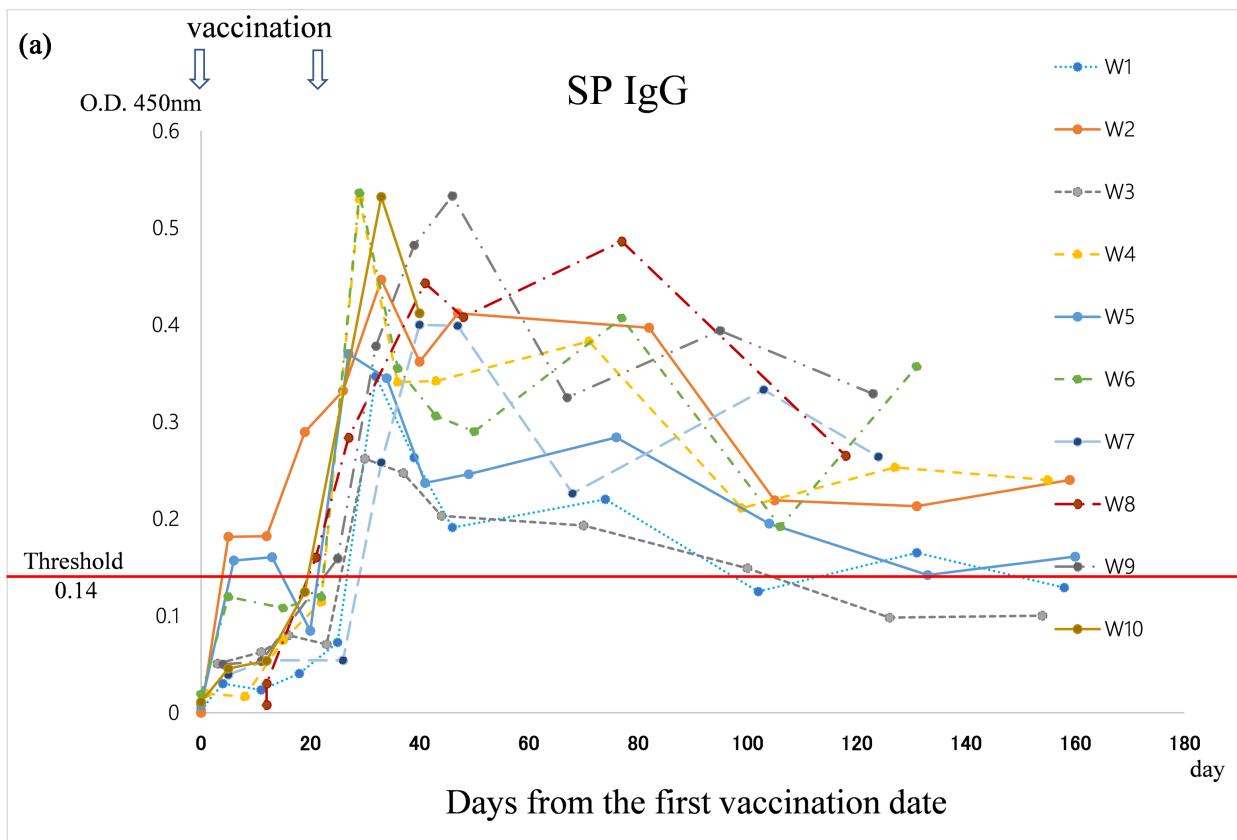
Figure 2. This set of boxplots shows the peak titers for each antibody of all subjects.
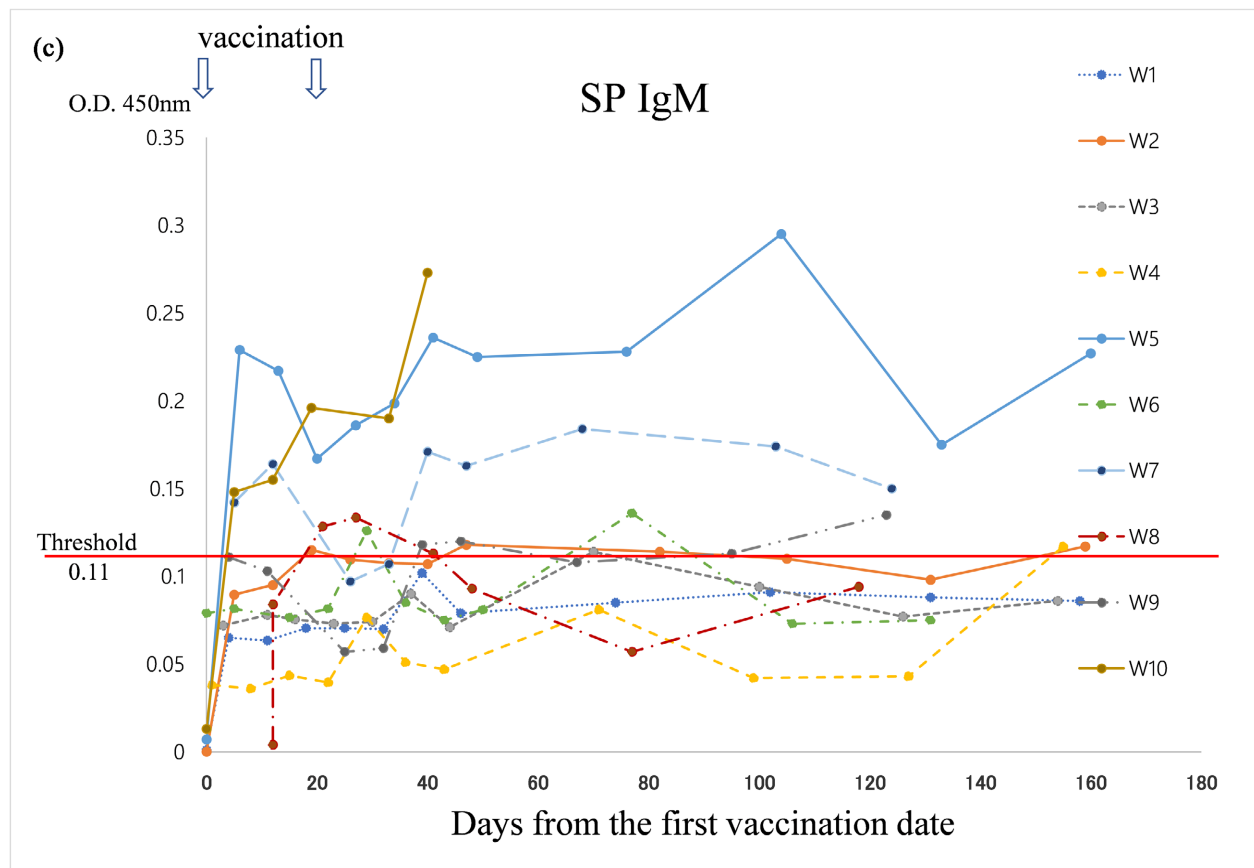
Figure 3. The antibody level after the first vaccination. The vertical axis is the OD value of ELISA, and the horizontal axis is the time since the first vaccination. The three figures show SP IgG (a), S1 IgG (b), and SP IgM (c). Solid lines (W2, W5, W10) are subjects with a history of allergies.ELISA, enzyme-linked immunosorbent assay; Ig, immunoglobulin; N, nucleocapsid protein; OD, optical density; S1, spike 1 protein; SP, spike 1 + 2 protein

showed a median of 0.236 for 3 people with allergic history and a median of 0.111 for 7 people without allergic history (**Figure 4(b)**).

4. Discussion

The majority of people got at least one shot at being influenced by the internet information, their work and their social life [4].

Vaccination against COVID-19 is followed by some spontaneously reported adverse events, and some of them are serious [5].

In this study, the titer of antibodies against COVID-19 after vaccination of COVID-19 was evaluated for healthy volunteers. We also investigated adverse events.

Elevation of SP IgG antibodies was observed in all cases. Several antibodies including S1 IgG, SP IgG, SP IgM, NP IgG, and NP IgM were analyzed in this study. Nucleocapsid protein (NP) of COVID-19 is not related to contact with host cells. Antibodies against NP have no effect to prevent infection with COVID-19. The NP antibody has been considered a marker of past infection with COVID-19.

(a)

SP IgG	side effects of grade2	
	n = 5	n = 5
Min	0.258	0.370
Median	0.347	0.447
Max	0.530	0.536

S1 IgG	side effects of grade2	
	n = 5	n = 5
Min	0.342	0.560
Median	1.077	1.059
Max	1.253	1.442

SP IgM	side effects of grade2	
	n = 5	n = 5
Min	0.077	0.111
Median	0.102	0.126
Max	0.164	0.273

(b)

SP IgG	history of allergic disease	
	n = 7	n = 3
Min	0.258	0.370
Median	0.378	0.447
Max	0.536	0.532

S1 IgG	history of allergic disease	
	n = 7	n = 3
Min	0.342	0.560
Median	1.107	0.732
Max	1.442	1.059

SP IgM	history of allergic disease	
	n = 7	n = 3
Min	0.077	0.118
Median	0.111	0.236
Max	0.164	0.273

Antibody levels were divided into two groups and analyzed by Mann-Whitney U test.

Figure 4. (a) Peak titers of each antibody with and without side effects of grade 2 or higher; (b) Peak titers of each antibody with and without a history of allergic disease.

In this study, elevation of S1 IgG and SP IgG antibodies was observed in all patients. S1 IgG and SP IgG peaked at about 30 days and then decreased in all cases. Usually, following infection with viruses, IgM is produced within 1 to 2 weeks, and IgG is produced several months later. However, it has been reported that IgG increased faster than IgM in COVID-19 infections [6]. One of the causes

has been suggested to be the involvement of cross-immunity against COVID-19 in immunological memory after infection with seasonal human coronavirus [7]. There is also a report that IgM antibody after vaccination does not increase in non-infected persons compared with pre-infected persons [8]. Further research is needed on IgM trends after COVID-19 infection.

The S1 IgG antibody, which is most involved in protection against infection, decreased over time in all cases from around 2 months, and decreased below the cutoff value in 3 months. This is recognized worldwide, and booster vaccinations have begun around the world.

In this study, IgG antibodies exceeded the cutoff in all cases, regardless of the underlying disorder, age, or presence or absence of side effects. Although there are few cases, we investigated whether there was a difference in the antibody elevation peak depending on the background factors. The peak value of IgG was not strongly associated with background factors, but the elevation of SP IgM may be associated with post-vaccine side effects and a history of allergic disease (Figure 4). Elissavet *et al.* reported that persons who experienced fever or adverse events after vaccination had higher IgG levels but did not mention a relationship with IgM [9]. Much remains unclear regarding how IgM behaves with COVID-19, but the elevation of IgM may be a marker for side effects, and this seems to be an important finding that requires future validation.

Although this was a small study of healthy volunteers in a single facility, few studies involve frequent measures of antibody titers after vaccination. In this study, changes in antibody titers for each patient were clarified. Elevated S1 IgG was observed in all healthy cases, and side effects after vaccination might be associated with elevated SP IgM. We hope that the results of this research will lead to further research on COVID-19.

Limitation

It has been discussed that serologic tests can have various false-positives and false-negatives due to several reasons [10]. Even if serologic tests are performed, various other reasons can affect the immune response and a sole test is not a golden ideal [11]. Since this study has a small number of subjects, it is necessary to investigate with an increased number of subjects in the future.

5. Conclusion

S1IgG and SPIgG exceeded the cutoff in all subjects after vaccination but decreased below the cutoff in all subjects within 4 months, regardless of side effects or allergic history. On the other hand, elevated SPIgM was suspected to be related to side effects and history of allergies, and cases with persistent elevation of SPIgM were observed.

Author Contributions

Hiromi Nagashima was responsible for writing this manuscript and the data

analysis. Shinji Chiba, Shinnosuke Oura, Itaru Fujimura, Masachika Akiyama, Yu Utsumi, were in charge of the collection of eligible patients. Hironori Sakai, Wataru Hojo, Takuya Iwabuchi were responsible for the antibody analysis. Makoto Maemondo was a chief investigator and was responsible for the coordination of the study.

Institutional Review Board Statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee at Iwate Medical University School of Medicine (MH2020-052).

Informed Consent Statement

Written informed consent has been obtained from the subject(s) to publish this paper.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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