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【 ORIGINAL ARTICLE 】

Antibody Responses to the BNT162b2 mRNA Vaccine in Healthcare Workers in a General Hospital in Japan: A Comparison of Two Assays for Anti-spike Protein Immunoglobulin G

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Abstract:

Objective This study assessed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibody responses to the BNT162b2 mRNA vaccine in Japanese healthcare workers.

Methods In this prospective cohort study, participants received two doses of the BNT162b2 mRNA vaccine on days 0 and 21 and provided blood for anti-SARS-CoV-2 antibody testing before the first vaccine and on days 21 and 35 after vaccination. Anti-spike protein immunoglobulin G (S-IgG) was measured using Abbott and Fujirebio chemiluminescent immunoassays.

Patients One hundred healthcare workers (median age: 39 years old, interquartile range: 30-48 years old), including 6 who had been previously infected with SARS-CoV-2 and 3 individuals taking immunosuppressive drugs, participated in the study.

Results The S-IgG antibody titers (AU/mL) measured using both the Abbott and Fujirebio assays increased significantly ($p<0.001$) over time, both with a prevalence of 100% at 35 days after the first vaccination. The multivariate log-normal linear regression analysis indicated the effect of immunosuppressant medication using both the Abbott ($p=0.013$) and Fujirebio ($p=0.039$) assays on S-IgG levels after complete vaccination. Pearson's correlation coefficient between the Abbott and Fujirebio S-IgG results in all 300 samples collected before and after vaccination and 50 positive controls from patients with coronavirus disease 2019 were 0.963 [95% confidence interval (CI): 0.954-0.970, $p<0.001$] and 0.909 (95% CI: 0.845-0.948, $p<0.001$), respectively.

Conclusion The BNT162b2 mRNA vaccine was effective at increasing S-IgG levels in Japanese immunocompetent healthcare workers. The Fujirebio S-IgG assay showed high diagnostic accuracy, using the Abbott S-IgG assay as the reference test.

Key words: coronavirus disease 2019, immunoglobulin, neutralizing antibody, severe acute respiratory syndrome coronavirus 2, BNT162b2 mRNA vaccine, anti-spike protein immunoglobulin G

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Introduction

Coronavirus disease 2019 (COVID-19) is an acute respiratory disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which has become a public health and economic problem worldwide since December 2019, continuing through April 2021 (1).

To overcome this unprecedented pandemic, various vaccines against SARS-CoV-2 have been developed (2). In Japan, the government initiated the preliminary vaccination of healthcare workers (HCWs) in February 2021 with

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BNT162b2 mRNA vaccine manufactured by Pfizer-BioNTech. The vaccine against SARS-CoV-2 produces antibodies that target the spike protein on the surface of the virus, thereby preventing infection and severe disease (3-5). Epidemiological studies are required to assess the effectiveness of vaccines for protection against infection and disease, but they take a long time to perform and cannot assess susceptibility to SARS-CoV-2 infection at the individual level.

To quickly assess the effect of a vaccine, neutralizing antibodies can be measured as an indicator of humoral immunity to determine the immunogenicity of the vaccine (6, 7), and cellular immunity can be checked (8, 9). In the early stages of vaccination, studies from other countries, including Japan, have reported that neutralizing antibodies increase after vaccination (6, 7). However, it is difficult to measure neutralizing antibody titers in general hospitals due to the complexity of the measurement. Various antibody assays have been developed in other countries (10-13). Studies from other countries have reported that neutralizing antibodies are mainly anti-spike protein immunoglobulin G (S-IgG), and the ARCHITECT SARS-CoV-2 IgG Quant, which measures S-IgG and is marketed by Abbott (Abbott Park, Chicago, USA), correlates with neutralizing antibodies (14, 15). A set of Japanese assays, including for S-IgG, was recently developed and launched by Fujirebio (Tokyo, Japan) as a commercially available antibody test that can be performed under standard laboratory safety conditions without complicated processes.

In the present study, to verify the immunogenicity of the BNT162b2 mRNA vaccine in the Japanese population, we measured the S-IgG levels using the Abbot and Fujirebio assays before and after vaccination. In addition, to validate the performance of the Fujirebio assay, we assessed the antibody response using the Fujirebio assay for S-IgG, anti-spike protein immunoglobulin M (S-IgM), and anti-nucleocapsid protein IgG (N-IgG) before and after vaccination and in positive and negative controls.

Materials and Methods

Study design and setting

This single-center prospective observational study was conducted at Shonan Fujisawa Tokushukai Hospital, Kanagawa, Japan. In Japan, the BNT162b2 vaccine was the first SARS-CoV-2 vaccine to become available, which is recommended to be administered intramuscularly through two doses, three weeks apart. In accordance with the Japanese government-led vaccination schedule, vaccination of the general public using the BNT162b2 vaccine began in February 2021 for HCWs in a limited number of designated hospitals. In our hospital, vaccination of HCWs was started in April 2021, and all 1,033 HCWs who wished to be vaccinated completed their second dose in May 2021.

Participants

HCWs at our hospital who were ≥20 years old and wished to be vaccinated were eligible for enrollment. Those who had a history of SARS-CoV-2 infection were allowed to participate, provided they had completed the isolation period and had clinically recovered. Potential participants were excluded if they met any of the following conditions: 1) had already received a COVID-19 vaccine, 2) were in the active phase of COVID-19, 3) did not wish to be vaccinated of their own choice, or 4) did not provide their informed consent. One hundred consecutive vaccinees were enrolled in this study from April to May 2021 and were followed up until June 2021. The target sample size of 100 was based on feasibility in terms of the budget and workload. All participants were asked if they were on immunosuppressive therapy or cytotoxic anticancer drugs, as these drugs might affect the antibody response.

As positive controls, we used 50 surplus serum samples from patients with COVID-19 admitted to our hospital between April 2020 and December 2020, who were confirmed to have had SARS-CoV-2 infection by real-time reverse transcription-polymerase chain reaction or loop-mediated isothermal amplification on nasopharyngeal or pharyngeal swabs or saliva specimens. Fifty samples were randomly selected, using a random number generator, from 298 samples collected from different patients in the convalescent phase, 3 to 6 weeks after the disease onset.

As negative controls, we used 50 surplus serum samples collected during the annual medical checkups of medical staff at our hospital. The checkups were conducted between June 2020 and July 2020, a few months after the first wave of the COVID-19 pandemic and before the development of any vaccine against SARS-CoV-2 in Japan. Fifty samples were randomly selected from among 800 available, using a random number generator, excluding samples from staff previously infected with SARS-CoV-2 and samples that had tested positive for N-IgG in a previous study (16).

Antibody measurement

We collected sera from participating HCWs within the week before vaccination (allowance range: -7 days) and 21 days (±2 days) and 35 days (±2 days) after the first vaccination. Collected serum that could not be measured on the day of collection and surplus serum samples as both positive and negative controls were frozen and stored below -80 °C until use.

At our hospital, we measured antibodies against SARS-CoV-2 using SARS-CoV-2 IgG II Quant, ARCHITECT SARS-CoV-2 IgG, and ARCHITECT SARS-CoV-2 IgM (Abbott). The ARCHITECT SARS-CoV-2 IgG II Quant is a chemiluminescent microparticle immunoassay (CMIA) used for the qualitative and quantitative determination of IgG antibodies to SARS-CoV-2 in human serum and plasma using the ARCHITECT i System. In the antibody test, the SARS-CoV-2 antigen-coated paramagnetic microparticles bind to

the IgG antibodies that attach to the virus spike receptor-binding domain in human serum and plasma samples. The manufacturer-recommended positivity cut-off of Abbott S-IgG is 50 AU/mL. Architects SARS-CoV-2 IgG and IgM are semi-quantitative CMIA assays that were designed to detect IgG and IgM antibodies against the nucleocapsid and spike protein, respectively, of SARS-CoV-2 in serum and plasma samples using the ARCHITECT i System. The results of Abbott N-IgG and Abbott S-IgM were reported as an index [ratio of the chemiluminescent signal between the samples and a calibrator (S/C)], with manufacturer-recommended positivity cut-off index values of 1.4 S/C and 1.0 S/C, respectively.

At the Fujirebio laboratory, the SARS-CoV-2 S-IgG, SARS-CoV-2 N-IgG, and SARS-CoV-2 S-IgM assays (Fujirebio), which are qualitative and quantitative chemiluminescent enzyme immunoassays, were performed using a LUMIPULSE L2400 analyzer, which is a fully automated chemiluminescent enzyme immunoassay system. The frozen specimens were then transported under temperature-controlled conditions. The manufacturer-recommended positivity cut-off values of Fujirebio S-IgG, Fujirebio N-IgG, and Fujirebio S-IgM are 1.0 AU/mL, 1.0 AU/mL, and 1.0 C.O.I. (cut-off index), respectively.

Ethical approval and data collection

The procedures were performed in accordance with the Declaration of Helsinki. The study protocol was approved by the Tokushukai Group Ethics Committee (approval no. TGE01697-008). Written informed consent was obtained from all HCWs prior to participation. However, the ethics committee waived the requirement for informed consent from patients with COVID-19 in the positive control group and HCWs in the negative control group because leftover samples were used. Clinical information was obtained from the electronic medical records and medical interviews of all participants.

Statistical analyses

Data analyses and visualization were performed using the R statistical software program (R Foundation for Statistical Computing, Vienna, Austria; https://www.R-project.org/). Continuous variables are expressed as the median with the interquartile range (IQR), as are antibody titers. The Steel-Dwass method was used for non-parametric multiple comparisons of the antibody titer of the time series. The Mann-Whitney U test was used for non-parametric multiple univariate comparisons between the two groups. Categorical variables are presented as numbers (percentages). Correlations were tested using Pearson's correlation coefficient. The agreement between different measurements of the same type was estimated using the kappa coefficient. A multivariate log-normal analysis was used to identify significant predictors of high antibody titers after complete vaccination. The selection of variables was based on the previous literature (17, 18) and included age, sex, history of SARS-CoV-2 infection, immunosuppressant medication, smoking history, and alcohol consumption. Two-sided p values <0.05 were considered statistically significant.

Table 1. Participants' Characteristics.

Variables	Participants (N=100)
Sex, male; n (%)	36 (36%)
Age (years), median [IQR]	39 (30-48)
Height (cm), median [IQR]	161.5 (156.0-169.3)
Weight (kg), median [IQR]	57.3 (49.0-67.0)
Body mass index, median [IQR]	21.5 (19.8-24.0)
Smoking history, n (%)	
Never	75 (75%)
Former or current	25 (25%)
Alcohol consumption frequency, n (%)	
None	54 (54%)
Less than 2 times per week	28 (28%)
More than 3 times per week	18 (18%)
Past history of COVID-19, n (%)	6 (6%)
Immunosuppressant drugs, n (%)	3 (3%)
Anticancer chemotherapy, n (%)	0 (0%)
Occupation, n (%)	
Nurse	39 (39%)
Clerical staff	27 (27%)
Laboratory medical technologist	18 (18%)
Doctor	13 (13%)
Pharmacist	3 (3%)

Results

Participants

A total of 100 Japanese HCWs were enrolled in this study. The study participants included 36 men and 64 women, with a median age of 39 (IQR: 30-48) years old, including 39 nurses, 27 clerical staff, 18 laboratory medical technologists, 13 doctors, and 3 pharmacists. Six of the participants had previously been diagnosed with COVID-19 based on clinical symptoms and a positive SARS-CoV-2 PCR test at a median of 2 months (range: 2 weeks to 13 months) prior to enrolment. Three participants with no history of COVID-19 were on immunosuppressive therapy: two were regular with their immunosuppressive medications, including one on a combination of corticosteroids and benralizumab for asthma and eosinophilic sinusitis and one on a combination of azathioprine, mesalazine, and golimumab for ulcerative colitis; the other was being temporarily treated with corticosteroids for pharyngitis and laryngeal edema by an otolaryngologist from 13 to 17 days after the first vaccination. None of the participants were taking anticancer drugs. All 100 participants received a second dose of the vaccine 21 days after the first dose. The participants' characteristics are shown in Table 1.

Table 2. Antibody Titer and Seropositivity According to the Assay and the Timing of Sample Collection.

Assay	Before the first vaccination (N=100)	21 days after the first vaccination (N=100)	35 days after the first vaccination (N=100)
Abbott S-IgG			
Median [IQR] (AU/mL)	1.45 [0.10-3.58]	1,145.3 [767.6-2,093.7]	18,874 [12,679-25,456]
Seropositive, n (%)	5 (5%)	99 (99%)	100 (100%)
Fujirebio S-IgG			
Median [IQR] (AU/mL)	0.1 [0.1-0.1]	16.85 [9.475-27.75]	321.6 [200.2-447.1]
Seropositive, n (%)	5 (5%)	100 (100%)	100 (100%)
Fujirebio S-IgM			
Median [IQR] (C.O.I.)	0.2 [0.1-0.3]	1.25 [0.7-2.3]	2.85 [1.7-4.55]
Seropositive, n (%)	3 (3%)	65 (65%)	89 (89%)
Fujirebio N-IgG			
Median [IQR] (AU/mL)	0.0 [0.0-0.0]	0.0 [0.0-0.0]	0.0 [0.0-0.0]
Seropositive, n (%)	4 (4%)	4 (4%)	4 (4%)

C.O.I.: cutoff index, N-IgG: anti-nucleocapsid protein immunoglobulin G, S-IgG: anti-spike immunoglobulin G, S-IgM: anti-spike immunoglobulin M, IQR: interquartile range

Antibody positivity after vaccination

Blood samples were collected 3 times as specified from all 100 participants after they had received both doses of the vaccine. The results for each of the four assays according to collection time are shown in Table 2. The concordance between the Abbott S-IgG and Fujirebio S-IgG results in all 300 samples before and after vaccination was 99.7% [299/300, 95% confidence interval (CI): 98.1-99.9%], and the kappa coefficient was 0.99 (95% CI: 0.98-1.00).

Prior to vaccination, five of the participants who had a history of SARS-CoV-2 infection were shown to be positive using both the Abbott S-IgG and Fujirebio S-IgG assays. The remaining previously infected participant developed COVID-19 two weeks before the first blood draw and tested negative prior to vaccination. Fujirebio N-IgG was positive in four participants before vaccination, all of whom had a history of SARS-CoV-2 infection. Among the previously infected participants, those infected 13 months and 2 weeks before enrollment were negative for N-IgG using the Fujirebio assay before vaccination. Three participants were positive for S-IgM using the Fujirebio assay before vaccination, of whom two had a history of SARS-CoV-2 infection (one and seven months before enrolment).

Antibody titer changes before and after vaccination and correlation between two assays of anti-spike IgG

Temporal dynamic changes in antibody levels and the median titer with the IQR for each of the 4 assays among the 100 participants are shown in Table 2. The titer of Abbott S-IgG, Fujirebio S-IgG, and Fujirebio S-IgM increased significantly over time ($p<0.001$). In contrast, the Fujirebio N-IgG titer did not change markedly over time (Fig. 1).

There was a discrepancy between the Abbott S-IgG and Fujirebio S-IgG results in only 1 sample, which was collected 21 days post-vaccination in a participant with immunosuppression, and both values were close to the cut-off values. The Abbott S-IgG result was negative (22.7 AU/mL), while the Fujirebio S-IgG result was weakly positive with a cut-off titer value of 1.0 AU/mL. The Pearson's correlation coefficient between the Abbott S-IgG and Fujirebio S-IgG results in all 300 samples collected before and after vaccination was 0.963 (95% CI: 0.954-0.970, $p<0.001$) (Fig. 2).

Factors contributing to antibody titers of anti-spike IgG 35 days after the first vaccination

The S-IgG levels were significantly higher in the 6 previously infected participants than in the 91 uninfected, non-immunosuppressed participants before and 21 days after the first vaccination using both the Abbott and Fujirebio assays ($p<0.001$) and 35 days after the first vaccination using the Fujirebio assay ($p=0.009$), but not using the Abbott assay ($p=0.16$). The S-IgG levels were significantly lower in the 3 participants who were taking immunosuppressant medication than in the 91 uninfected, non-immunosuppressed participants at 35 days after the first vaccination (Abbott, $p=0.005$ and Fujirebio, $p=0.004$). Among all 100 participants, considering the antibody titer of both Abbott and Fujirebio assays 35 days after the first vaccination, no significant difference was found in smoking history ($p=0.47$ and $p=0.28$, respectively) or alcohol consumption more than 3 times per week ($p=0.47$ and $p=0.78$, respectively). The S-IgG antibody titer 35 days after the first vaccination in women (n=64) was a median of 355.4 (IQR: 204.0-478.0) AU/mL for the Fujirebio assay and a median of 20,585 (IQR: 12,679-27,802) AU/mL for the Abbott assay, whereas men (n=36) had a median of 289.1 (IQR: 198.1-402.6) AU/mL and a median of 17,629 (IQR: 12,658-22,189) AU/mL for the Fujirebio and Abbott assays, respectively. Thus, the S-IgG antibody titer in women tended to be higher than that in men, but the difference was not significant in either assay ($p=0.19$ and $p=0.15$, respectively).

The multivariate log-normal linear regression analysis in-

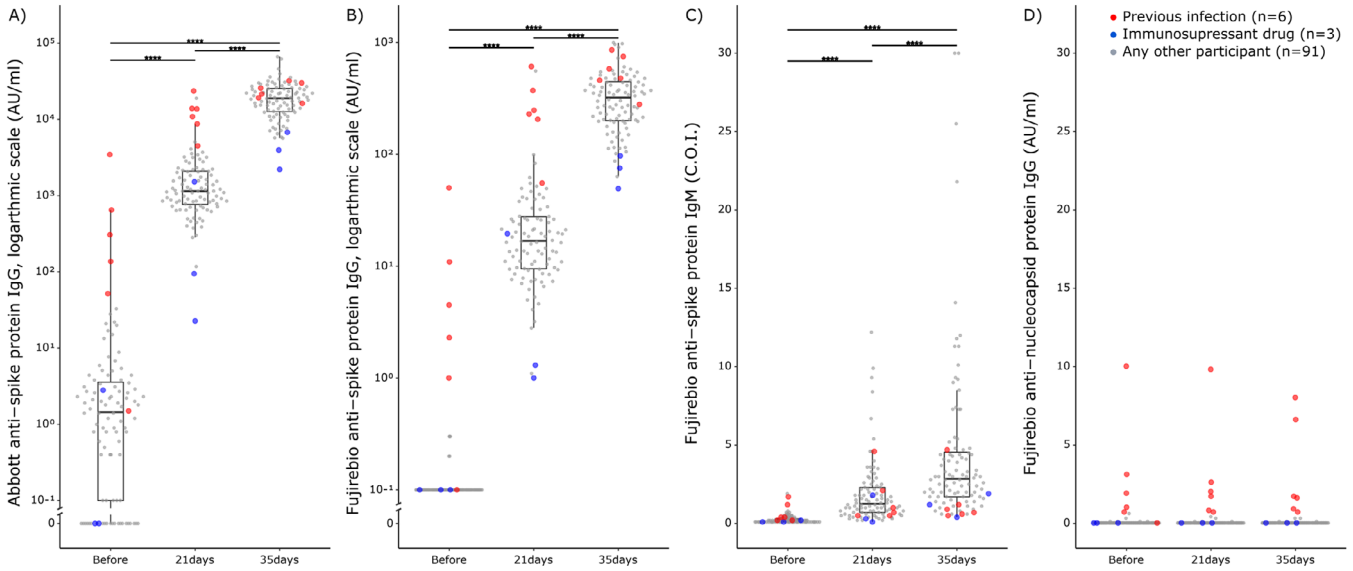

Figure 1. **Beeswarm and box plots show the temporal dynamic changes in antibody levels with each of the 4 assays among 100 participants who received both doses of the BNT162b2 mRNA SARS-CoV-2 vaccine. A) Abbott anti-spike protein immunoglobulin G (IgG); B) Fujirebio anti-spike protein IgG; C) Fujirebio anti-spike protein immunoglobulin M (IgM); D) Fujirebio anti-nucleocapsid protein IgG. S-IgG antibody titers in A) and B) are expressed on a logarithmic scale. The red dots are the six participants infected with SARS-CoV-2, and the blue dots are the two participants on immunosuppressant drugs. Both Abbot and Fujirebio anti-spike protein IgG and Fujirebio anti-spike protein IgM showed an increase over time. The Steel-Dwass test was used to compare antibody titers in time-series data. (**** p<0.001). Box plots display the median values with the interquartile range (IQR) (lower and upper hinge) and ±1.5-fold the IQR from the first and third quartile (lower and upper whiskers).**

dicated the effect of immunosuppressant medication using both the Abbott (p=0.013) and Fujirebio (p=0.039) assays and previous infection (p=0.008) using only the Fujirebio assay on S-IgG levels, 35 days after the first vaccination (Table 3).

Performance of the Fujirebio assays in COVID-19 patients

In the positive controls, all 50 samples were positive (100%) using all 6 assays (Abbott and Fujirebio S-IgG, S-IgM, and N-IgG). In the negative controls, all 50 samples were negative (100%) for all 6 assays (Abbott and Fujirebio S-IgG, S-IgM, and N-IgG). All antibody median titers with IQR are shown in Table 4.

In the 100 control samples (50 positive and 50 negative controls), the sensitivity, specificity, positive predictive value, and negative predictive value of Fujirebio S-IgG, S-IgM, and N-IgG were all 100%. The concordance between Abbott S-IgG and Fujirebio S-IgG, Abbott S-IgM and Fujirebio S-IgM, and Abbott N-IgG and Fujirebio N-IgG was 100% (100/100), and the kappa coefficients were all 1.0. Among the 50 positive controls, the Pearson's correlation coefficient between Abbott S-IgG and Fujirebio S-IgG was 0.909 (95% CI: 0.845-0.948, p<0.001; Fig. 3).

Discussion

The S-IgG levels of the 100 participants, including 6 previously infected individuals who received a second dose of the vaccine 21 days after the first dose, increased over the first 35 days after vaccination. All participants showed positive S-IgG levels in both the Abbott and Fujirebio assays 35 days after the first vaccine dose, thereby confirming the immunogenicity of this vaccine in the Japanese population. The kappa value of the 2 S-IgG antibody assays was 0.992, and the correlation coefficient was 0.96 using all 300 specimens collected from the participants before and after vaccination, with a kappa value of 1.0, and a correlation coefficient of 0.91 for the control specimens, including the specimens from patients in the convalescent phase of COVID-19, confirming that the newly launched Fujirebio S-IgG assay was highly correlated with the Abbott S-IgG assay. A multivariate log-normal linear regression analysis showed the effect of immunosuppressant medication using both the Abbott and Fujirebio assays on S-IgG levels, 35 days after the first vaccination.

There have been several reports on the evaluation of antibody responses after the BNT162b2 mRNA vaccination using assays developed in Western countries (14, 15, 17-19). In Japan, several antibody testing assays against SARS-CoV-

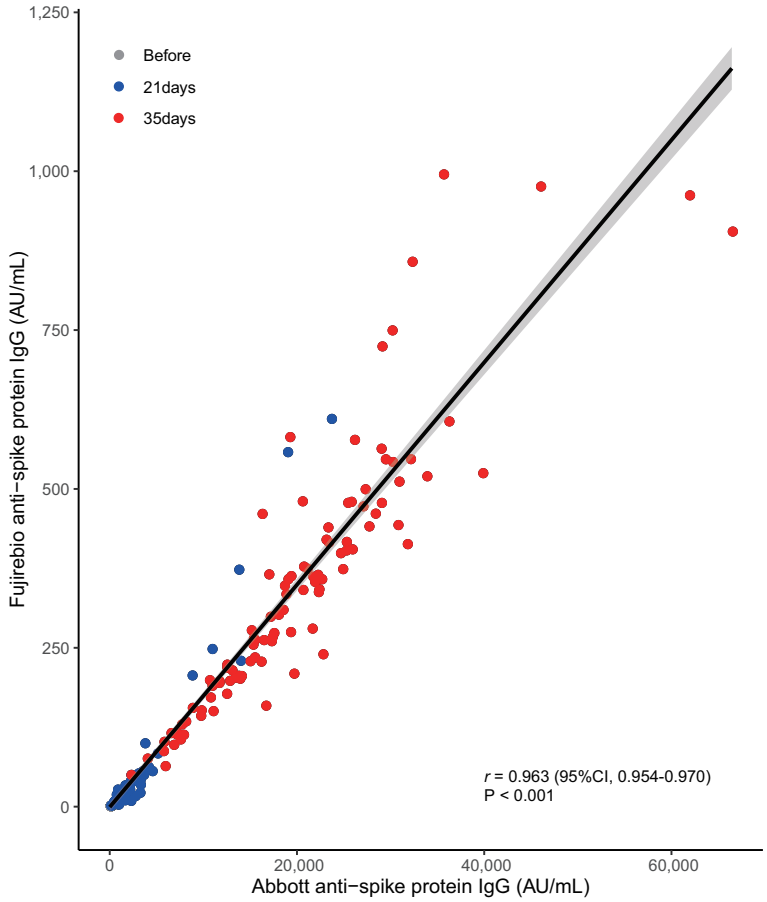

Figure 2. **Scatter plot examining the relationship between the Abbott and Fujirebio anti-spike protein immunoglobulin G (IgG) titers of serum samples of the 100 participants who received 2 doses of the BNT162b2 mRNA SARS-CoV2 vaccine, including regression line (black dot: before first vaccination, red dot: 21 days after first vaccination, blue dot: 35 days after first vaccination). The Pearson correlation coefficient was 0.963 (95% confidence interval: 0.954-0.970, p<0.001), indicating a highly positive association.**

Table 3. **Multivariate Log-normal Analysis of Factors Contributing to Anti-spike Immunoglobulin G Antibody Level 35 Days after the First Vaccination.**

	Fujirebio assay			Abbott assay		
Variables	**Value**	**t value**	**p value**	**Value**	**t value**	**p value**
Age	-0.81	-0.44	0.66	26.3	0.27	0.79
Sex: Female	70.12	1.52	0.13	4,225.2	1.71	0.09
Previous SARS-CoV-2 infection	227.10	2.73	0.008	4,000.9	0.90	0.37
Immunosuppressant medication	-261.03	-2.09	0.039	-16,897.3	-2.52	0.013
Smoking history	12.29	0.23	0.82	1,367.8	0.47	0.64
Alcohol consumption more than 3 times per week	29.36	0.56	0.58	3,293.6	1.17	0.25

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2

2 are commercially available at present for research purposes, none of which are being reimbursed by insurance in Japan yet (20). The principles of these assays range from a qualitative immunochromatographic assay to a quantitative chemiluminescent enzyme immunoassay, chemiluminescent immunoassay, electrochemiluminescence immunoassay, enzyme-linked immunosorbent assay, fluorescence immunoassay, and lateral flow assay (20). The globulin class of the antibody includes IgM, IgG, and total Ig, and the target antigens of these assays include the nucleocapsid protein, spike protein, and receptor-binding domain (RBD) of the spike protein (20). To our knowledge, this is the first report to evaluate humoral immune responses to the BNT162b2 mRNA vaccination and SARS-CoV-2 infection using a domestic assay developed and launched by Fujirebio in Japan, with the SARS-CoV-2 S-IgG, SARS-CoV-2 N-IgG, and SARS-CoV-2 S-IgM assays launched in April, July, and March 2021, respectively. SARS-CoV-2 S-IgG and SARS-

Table 4. Antibody Titer and Seropositivity in the 50 Positive and 50 Negative Controls According to the Assay.

Assay	Negative controls (N=50)	Positive controls (N=50)
Abbott S-IgG		
Median [IQR] (AU/mL)	2.0 [1.2-3.5]	6,089.7 [3,586.2-15,193.6]
Seropositive, n (%)	0 (0%)	50 (100%)
Abbott S-IgM		
Median [IQR] (S/C)	0.08 [0.06-0.12]	13.23 [5.88-20.29]
Seropositive, n (%)	0 (0%)	50 (100%)
Abbott N-IgG		
Median [IQR] (S/C)	0.03 [0.02-0.05]	7.58 [6.73-8.20]
Seropositive, n (%)	0 (0%)	50 (100%)
Fujirebio S-IgG		
Median [IQR] (AU/mL)	0.1 [0.1-0.1]	64.45 [32.15-156.68]
Seropositive, n (%)	0 (0%)	50 (100%)
Fujirebio S-IgM		
Median [IQR] (C.O.I.)	0.2 [0.1-0.2]	13.45 [6.6-23.98]
Seropositive, n (%)	0 (0%)	50 (100%)
Fujirebio N-IgG		
Median [IQR] (AU/mL)	0.1 [0.1-0.1]	19.75 [7.675-35.375]
Seropositive, n (%)	0 (0%)	50 (100%)

C.O.I.: cutoff index, N-IgG: anti-nucleocapsid protein immunoglobulin G, S-IgG: anti-spike immunoglobulin G, S-IgM: anti-spike immunoglobulin M, IQR: interquartile range, S/C: the ratio of the chemiluminescent signal between the samples and a calibrator

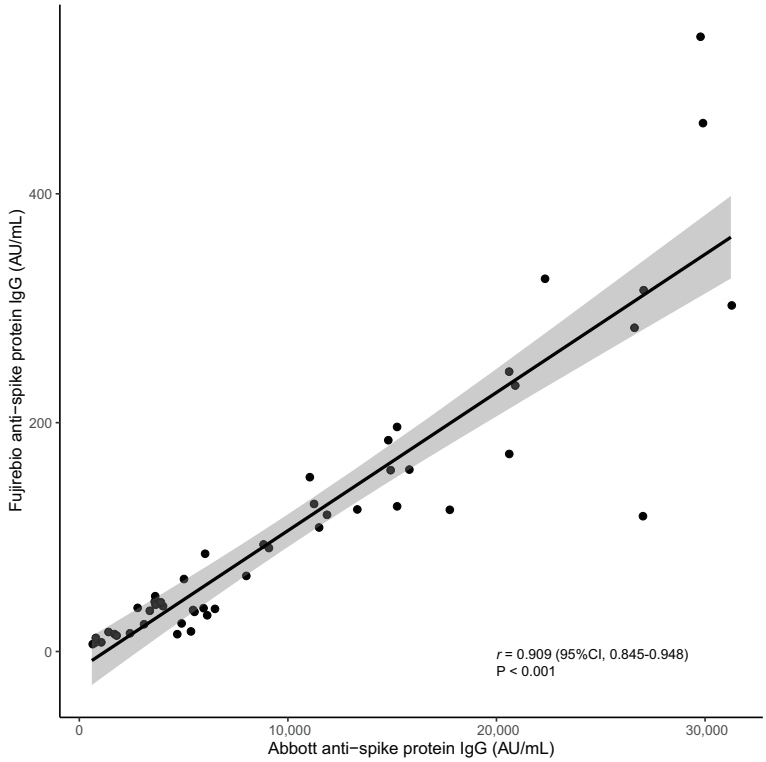

Figure 3. Scatter plot examining the relationship between Abbott and Fujirebio anti-spike protein immunoglobulin G (IgG) assays in serum samples of 50 COVID-19 patients in the convalescent phase (positive controls). The Pearson correlation coefficient was 0.909 (95% confidence interval: 0.845-0.948, p<0.001), indicating a highly positive association.

CoV-2 N-IgG were accepted for testing at SRL, in May and October 2021, respectively, and are still in use as of November 2021. One participant with no history of SARS-CoV-2 infection tested positive for S-IgM before vaccination and

negative for N-IgG in all three blood samples, suggesting that the positive result for S-IgM before vaccination was a false positive. As previously reported, N-IgG was positive only in previously infected individuals, and no change was observed before and after vaccination (19). The negative N-IgG results in the 2 participants with a COVID-19 onset 13 months and 2 weeks before enrolment, were assumed to have been due to elevated antibody titers that disappeared over time and not yet having produced antibodies against SARS-CoV-2, respectively. The combination of S-IgG and N-IgG can be used to estimate the SARS-CoV-2 infection status and evaluate immunogenicity after vaccination, although a window period immediately after infection or a long period after infection may result in negative results (19).

In previous studies, immunogenicity has been shown to decrease depending on the age, smoking habit, alcohol consumption, and sex (men); however, in the present study, there was no significant difference in the results of a multivariate log-normal linear regression analysis. One possible explanation for this is the small number of cases exposed to these conditions, which may be attributable to a lack of power.

A recent study evaluated the conservative IgG spike-RBD (S-RBD) level (Abbott assay) as a surrogate measure of antibody neutralization and found that at or above 4,160 AU/mL, the IgG (S-RBD) titer estimates consistently corresponded to a 0.95 probability of the plaque reduction neutralization test proportion at a 50-stringency threshold for 1/250 dilution (15, 21). In the present study, all 98 immunocompetent participants (including 1 with temporary corticosteroid use) had an S-IgG value ≥4,160 AU/mL on Abbott's assay; however, 2 participants, both of whom were immunosuppressed with regular use of immunosuppressant medication, had Abbott's S-IgG value <4,160 AU/mL 35 days after the first vaccination. This suggests that the vaccination of immunosuppressed individuals may not result in the production of sufficient neutralizing antibodies.

Several limitations associated with the present study warrant mention. First, because the observation period was short, the duration of high antibody levels following vaccination could not be determined. Second, we were unable to measure neutralizing antibodies; therefore, the direct correlation between Fujirebio S-IgG and neutralizing antibodies remains unknown. Finally, although other vaccines have been launched, the present study showed the response to only one type of mRNA vaccine.

As increases in S-IgG are correlated with the neutralizing antibody titer following vaccination (6), S-IgG is assumed to be a surrogate marker of protection against infection (22-24). Individuals with high S-IgG titers are less susceptible to SARS-CoV-2 infection and less likely to become severely ill than those with lower values (5, 22-24). In the future, the correlation between the long-term changes in the S-IgG antibody titer and COVID-19 susceptibility and severity should be investigated. In addition, only three participants in the present study were on immunosuppressant medication, of whom two had a background that could be considered immunocompromised. In general, the lower the immunocompetence, the greater the need for vaccines, but it is hypothesized that immunosuppressed individuals would experience a lower immune response to vaccines and thus a lower antibody titer. In the future, it will be necessary to confirm changes in S-IgG titers after vaccination in immunocompromised individuals in the Japanese population, including older adults, patients on anticancer or immunosuppressive drugs, and dialysis patients.

The authors state that they have no Conflict of Interest (COI).

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