Rikei Kozakai, Akira Kushida, Paul Franck Adeyissimi Adjou Moumouni, Sadatsugu Okuma, Kazuya Takahashi, Kuniko Hoshi, Yuri Sato, Mizue Takahashi, Nodoka Chida, Mei Takahashi, Shukuko Iwabuchi, Yoshihiko Izumi, Kana Fukami, Shuji Nakashiro, Hisashi Nojima and Shinichiro Takahashi*

Assessment of COVID-19 mRNA vaccination titer and side effects in healthy volunteers

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Abstract

Objectives: An effective vaccine against SARS-CoV-2 is essential to mitigate the COVID-19 pandemic. In these several months, a number of groups have started to report humoral responses and side effects after BNT162b2 vaccinations. Although these reports demonstrate the safety and efficacy, further studies are warranted to verify these findings. Here we examined the levels of SARS-CoV-2 antibodies in Japanese healthy volunteers who underwent BNT162b2 vaccine, to assess the humoral responses and side effects.

Methods: Forty-one healthy volunteers' samples were used for the measurement of SARS-CoV-2 antibodies with chemiluminescent assays against the Receptor Binding Domain (RBD) of the virus. We also measured the side effects of the vaccination.

Results: Although the levels of IgM varied, all participants were seronegative for IgM and IgG before vaccination, and both IgM and IgG were significantly increased after the vaccinations. We further analyzed the humoral responses in relation to age, and found that the IgG levels for 14 days and 35 days, and IgM levels for 14 days after vaccination showed clear declining trends with age. Commonly reported side effects in the participants were sore arm/pain

(90.0%) after the first dose, and generalized weakness/fatigue (70.0%), fever (57.5%), and sore arm/pain (90.0%) after the second dose.

Conclusions: BNT162b2 vaccination generates sufficient production of IgG especially after the second dose, though the response decreases age-dependently. The high frequencies of generalized weakness/fatigue, fever, and sore arm/pain were not negligible, especially after the second dose. This may be associated with the age characteristics of the population.

Keywords: BNT162b2 vaccine; humoral response; SARS-CoV-2; side effects; vaccination.

Introduction

As of the end of October 2021, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has infected over 242 million individuals worldwide, resulting in more than 4.92 million deaths. Many vaccines are now being employed to prevent the novel coronavirus disease 2019 (COVID-19) caused by SARS-CoV-2 infection, including RNA vaccines [1]. BNT162b2 (Comirnaty; BioNTech/Pfizer) is a lipid nanoparticle-formulated, nucleoside-modified mRNA vaccine for the prevention of COVID-19 [2]. The BNT162b2 vaccine was investigated in the present study because it was the only vaccine approved by the Ministry of Health, Labour and Welfare, Japan, when the study protocol was planned. In clinical studies of BNT162b2, the geometric mean titer of neutralizing antibodies associated with 50% neutralization increased after the first dose, and the vaccine provided high levels of protection against infection and severe disease after the second dose [3]. In a randomized, placebo-controlled clinical trial involving approximately 44,000 participants, immunization conferred 95% protection against COVID-19 [4]. Related mRNA vaccines also appear to be safe and effective [5, 6]. Recently, humoral responses in three volunteers after BNT162b2 vaccination were reported by Danese et al. [7]. They also reported that prominent humoral immune responses, especially

*Corresponding author: Shinichiro Takahashi, Division of Laboratory Medicine, Faculty of Medicine, Tohoku Medical and Pharmaceutical University, Sendai, Japan, Phone: +81-22-290-8889, E-mail: shintakahashi@tohoku-mpu.ac.jp

Rikei Kozakai, Kuniko Hoshi, Yuri Sato, Mizue Takahashi, Nodoka Chida, Mei Takahashi, Shukuko Iwabuchi and Yoshihiko Izumi, Department of Clinical Laboratory, Tohoku Medical and Pharmaceutical University Hospital, Sendai, Japan

Akira Kushida, Kana Fukami and Shuji Nakashiro, Product Planning Department, Fujirebio Inc., Tokyo, Japan

Paul Franck Adeyissimi Adjou Moumouni, Sadatsugu Okuma and Hisashi Nojima, R&D Strategy Planning Section, Fujirebio Inc., Tokyo, Japan

Kazuya Takahashi, Product Development Department Product Designing 4th Section, Fujirebio Inc., Tokyo, Japan

involving anti-SARS-CoV-2 IgG and IgA, were intensified by a second vaccination dose [7]. In these several months, a number of groups have started to report humoral responses and side effects after BNT162b2 vaccinations [8–10]. Although these reports demonstrate the safety and efficacy, further studies are warranted to verify these findings. The present study enrolled 41 healthy Japanese healthcare worker volunteers to determine the antibody production after BNT162b2 vaccination based on anti-SARS-CoV-2 titers. These titers were evaluated using Lumipulse platform, which is highly sensitive and fully automated assay system based on a chemiluminescent enzyme immunoassay (CLEIA) to specifically detect IgM and IgG, especially those recognizing the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein, which is involved in ACE2-mediated viral entry.

Materials and methods

Participants

The vaccination campaign for administration of 30 µg of BNT162b2 vaccine (Comirnaty; BioNTech/Pfizer, New York, NY, USA) began in Tohoku Medical and Pharmaceutical University Hospital on 15 March 2021. Forty-one healthy volunteers were enrolled in the present study. They comprised 31 female and 10 male healthcare workers who received a first BNT162b2 vaccine dose from March to April 2021, followed by a second identical dose 21 days later. All the blood samples were collected before 1–3 days before vaccination, two weeks after first vaccination, and two weeks after second vaccination (Figure 1A). Then the samples were centrifuged at 1,690×g for 10 min at room temperature, and the serum was separated for storage in two 1-mL aliquots at –80 °C. The study design was approved by the Ethics Committee at the Tohoku Medical and Pharmaceutical University Hospital in the 2020 fiscal year (2020-2-256).

Figure 1: Vaccination procedures, sampling and anti-SARS-CoV-2 antibody levels following vaccination.

(A) Schematic presentation of vaccination and sampling procedures. (B, C) Box plots of anti-SARS-CoV-2 antibody levels following vaccination with the BNT162b2 vaccine. (B) Anti-SARS-CoV-2 IgM at baseline, and at 14 and 35 days after the first vaccination in all participants. (C) Anti-SARS-CoV-2 IgG at baseline, and at 14 and 35 days after the first vaccination in all participants. Box plots display the median values with the interquartile range (lower and upper hinge) and ± 1.5 -fold the interquartile range from the first and third quartile (lower and upper whiskers). Data were analyzed using the Friedman test followed by pairwise Wilcoxon signed rank tests with Bonferroni correction. **** $p < 0.0001$.

Safety assessments

Safety assessments were monitored by solicited local and systemic adverse events, collected by a questionnaire. Items for symptoms and events were utilized and modified from the study by Kadali et al. [11]. The questionnaire was sent by e-mail to the participants enrolled in the study, and responses were collected until the middle of May 2021. Responses were obtained from 40 of the 41 healthy healthcare volunteers (Table 1) who received two doses of the BNT162b2 vaccine. For analysis of the questionnaire data, the responses were displayed as both percentage and number.

Antibody assays

All serum aliquots were thawed at the same time for analysis at the end of the study, and examined using SARS-CoV-2 S-IgG and SARS-CoV-2 S-IgM reagents (Fujirebio, Tokyo, Japan) for the *in vitro* quantitative determination of antibodies to the SARS-CoV-2 spike (S) protein receptor binding domain (RBD), according to the manufacturer's instructions. The levels of IgG or IgM were expressed in arbitrary units (AU)/mL or evaluated in accordance with a cut-off index (C.O.I.), respectively, which were calculated by quantification of the luminescence in the samples. The assay results are interpreted as reactive (>1.0 AU/mL or >1.0 C.O.I.; positive) and non-reactive (<1.0 AU/mL or <1.0 C.O.I.; negative) as shown in the manufacturer's instructions. Dilutional linearity experiments were performed with the samples; high concentrated serums were diluted using the anti-SARS-CoV2 antibody negative serums.

Statistical analysis

Humoral immune response data were statistically analyzed using Prism software (version 7.0; GraphPad Software Inc., La Jolla, CA, USA) or

R version 4.0.5 (R Foundation for Statistical Computing). The level of significance was set at $p \leq 0.05$. Correlations between Age and the levels of IgM or IgG were examined with Spearman's correlation test. Group differences in the antibodies' levels were first tested with Friedman or Kruskal–Wallis test and, when statistically significant, further investigated with the Wilcoxon signed-rank test or the Wilcoxon rank-sum test. For group comparisons, p-values were corrected using the Bonferroni or the Steel–Dwass method.

Results

Specificity of the antibody assays were confirmed using SARS-CoV-2 negative sera which were collected before 2019, resulting in 100% (1,011/1,011) for SARS-CoV-2 S-IgG reagents and 99.8% (1,056/1,058) for SARS-CoV-2 S-IgM reagents (Supplementary Figure S1A–B). Dilutional linearity was performed with samples which were within the working range and showed reasonable results (Supplementary Figure S1 C–D). The reproducibility tests of the kits resulted in reliable outcomes (Supplementary Table S1). The cumulative results for antibody production are shown in Figure 1, panels B–C. Although there were no obvious symptoms or history of COVID-19 infection, one of the 41 participants had a marginally increased level of SARS-CoV-2 IgM (2.1 C.O.I.) before vaccination (Figure 1B), which we considered to be within the variation of seronegative, and all subjects had baseline levels of SARS-CoV-2 IgG (Figure 1C).

At 14 days after the first vaccination, anti-SARS-CoV-2 IgM was modestly but significantly increased (average, 6.5-fold) (Figure 1B), while anti-SARS-CoV-2 IgG was more markedly increased (average, 93-fold) (Figure 1C). At 35 days after the first vaccination, anti-SARS-CoV-2 IgM was significantly increased (average, 19.5-fold) (Figure 1B), while anti-SARS-CoV-2 IgG was prominently enhanced (average, 2719-fold) (Figure 1C). We further analyzed the humoral responses in relation to age, and found that the IgG levels for 14 days and 35 days, and IgM levels for 14 days after vaccination showed clear declining trends with age, though an age difference was not seen in the IgM levels at 35 days after the vaccination (Figure 2 and Supplementary Figure S2). We further analyzed the differences between males and females, but no significant difference in both IgG and IgM titers were observed (data not shown).

Next, we assessed the side effects after vaccination (Figure 3 and Supplementary Table S2). Throughout the study, almost all participants (90% after the first dose;

Table 1: Demographic data of the study participants.

	Percentage, %	Number, n
Age group, years		
21–30	31.7	13
31–40	29.3	12
41–50	12.2	5
51–60	17.0	7
61–70	9.8	4
Gender		
Female	75.6	31
Male	24.4	10
Previous infection with SARA-CoV-2		
Infected	0.0	0
Not infected	100.0	41
Type of COVID-19 vaccine		
Pfizer BNT162b2 vaccine	100.0	41
Inoculated vaccine dose		
1st Dose	0.0	0
1st and 2nd Dose	100.0	41

Figure 2: Correlation of age and post-vaccination at day 14 (A, C) and day 35 (B, D) of anti-Spike IgM (A, B) or IgG (C, D) levels with superimposed the linear regression lines (with 95% CI).

The statistical methods of Spearman's rank correlation coefficient were used. p-Value is indicated. Linear regression lines, blue.

97.5% after the second dose) had symptoms (Figure 4A). The total number of side effects was significantly increased after the second vaccination, compared with the first vaccination (Figure 4B). We further analyzed

whether there were any differences in the numbers of side effects among the age groups, but no significant differences were found (Figure 4C and D, Supplementary Figure S3).

Figure 3: Summary of the side effects of the BNT162b2 vaccine after the first (black bars) and second (gray bars) doses of the vaccine. The data are shown as frequency (%).

Figure 4: Summary of the side effects of the BNT162b2 vaccine.

(A) Percentages for the presence (symptoms) or absence (no symptoms) of the side effects of the BNT162b2 vaccine reported by the participants after the first and second doses of the vaccine. (B) Box plots of total numbers of side effects in individual participants after the first and second doses. (C, D) Box plots of the total numbers of side effects in individual participants, categorized into five different age groups, at first dose (C), and second dose (D). Box plots display the median values with the interquartile range (lower and upper hinge) and ± 1.5 -fold the interquartile range from the first and third quartile (lower and upper whiskers). Data were analyzed using the Wilcoxon rank-sum test.

**** $p < 0.0001$.

Discussion

Side effects of the BNT162b2 vaccine have been reported by several groups [10–12]. In a study on 803 healthcare workers who received the BNT162b2 vaccine [11], 92.9% of the workers received the first and second doses, and were evaluated for side effects. The most common side effects of the vaccine were sore arm/pain (88.04%), generalized weakness/fatigue (58.89%), muscle pain/myalgia (45.70%), headache (45.48%), chills (36.60%), and fever (21.99%). In the present study conducted on Japanese subjects, the types of side effects did not differ significantly from those in the previous reports, but there were some differences in their

frequency. BNT162b2 was supposed to show a lower incidence and severity of systemic reactions, particularly in older adults [6, 10]. In the present study, however, we observed a clearly higher rate of fever (57.5%) after the second dose. In addition, there were no significant differences between side effects and age group (Figure 4C and D). These differences may have arisen from the sample size or generational differences; the relatively younger age of the population of volunteers enrolled in the present study (Table 1) compared with the other studies [10–12].

Published information on humoral responses to BNT162b2 remains limited. In the present study, we observed insufficient induction of both IgM and IgG titers

after the first vaccine dose (Figure 1). These results are consistent with a study showing that a single BNT162b2 vaccine dose in naïve subjects induced low levels of neutralizing antibodies [13, 14]. In the present study, the participants obtained high levels of anti-SARS-CoV-2 RBD IgG at 2 weeks after the second vaccination, consisting with the findings in previous reports [6, 15, 16]. These findings suggest that vaccination with both the first and second doses is necessary to achieve sufficient effects.

The present observation of low anti-SARS-CoV-2 RBD IgM titers after vaccination is consistent with the results from other groups [17, 18]. These findings suggest that the IgM antibody class may have limited importance for neutralization of SARS-CoV-2. We consider that IgG, but not IgM, is suitable for evaluating the response to vaccination, because of the high titers.

Although only a few data on the IgM antibody response after BNT162b2 vaccination are available, Beharier et al. [19] recently published an analysis of maternal-to-neonatal transfer of antibodies elicited by the BNT162b2 vaccine. In their analysis of 213 participants (vaccinated: $n=86$; infected: $n=65$; unvaccinated non-infected controls: $n=62$), the levels of anti-RBD IgM in unvaccinated non-infected controls varied from approximately 1 to 4 log₁₀ mean fluorescence intensity (MFI). However, most converged at <3.5, which we consider to be approximately equal to our quantitative unit C.O.I. We observed a substantially high SARS-CoV-2 IgM level in one participant (Figure 1B), and several cases above 4 log₁₀ MFI were similarly observed in the previous study [19]. In our separate experiment, 1,056 of 1,058 negative samples were below 1 C.O.I., indicating a specificity of 99.8% for this reagent (Supplementary Figure S1). IgM has been reported to have a non-specific response, as represented by (but not limited to) rheumatoid factor [20], therefore, the IgM assay, as in this reagent, may be more susceptible to interferences than the IgG assay. In fact, inhibition assay using anti-human IgM antibody resulted in a decrease in the measured values of this supposedly pseudo-high sample, suggesting that a non-specific reaction by IgM was the probable reason (data not shown).

We demonstrated that there was a significant inverse correlation between anti-RBD IgG and age (Figure 2) and the similar trend has reported [6, 8, 14]. Several factors have been demonstrated to affect anti-SARS-CoV-2 antibody titers. A study in the United States reported that mRNA vaccines generally showed lower virus-neutralizing responses in participants aged 65–85 years compared with those aged 18–55 years [6]. In Germany, Müller et al. [8] demonstrated that the SARS-CoV-2 spike-specific IgG titer was significantly lower in after both the first and second vaccinations in those aged ≥80 years. In the present study on Japanese subjects,

the results were similar to those previously reported in the United States and Germany. In contrast, Watanabe et al. [9] recently investigated an Italian study population and reported that higher waist circumference, smoking, hypertension, and longer elapsed time after second vaccination were associated with lower antibody titers. In their report [9], BMI, age, and sex were not related to antibody production. We consider that these discrepancies arise from differences in the population characteristics (age, weight, waist circumference tendency) of the healthcare workers in the different countries involved in the studies. It is also worth noting that antibody titers tend to decline rapidly following vaccination with kinetics that have not been completely clarified and may potentially be different in some populations [16].

In this study, we report for the first time the changes in antibody titers over time in Japanese patients before and after vaccination determined using the newly developed Lumipulse detection system. Although a variety of reagents have been used to measure anti-SARS-CoV-2 antibodies, the fact that the Lumipulse reagents were able to confirm the acquisition of antibodies after vaccination suggests that they can measure anti-SARS-CoV-2 antibodies appropriately. The Lumipulse reagents may be useful in such studies. Timely monitoring by employing these antibodies could be one of the efficient tools to conquer the ongoing COVID-19 pandemic.

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Ethical approval: Research involving human subjects complied with all relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration (as revised in 2013), and has been approved by the Ethics Committee at the Tohoku Medical and Pharmaceutical University Hospital in the 2020 fiscal year (2020-2-256).

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