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Original Article

Immunological responses following the third dose of the BNT162b2 SARS-CoV-2 vaccine among Japanese healthcare workers

Miku Tamura [a], Retsu Fujita [b], Tomoaki Sato [c], Ryohei Sato [d], Yasuyuki Kato [e], Mitsuaki Nagasawa [f], Tetsuya Matsumoto [g,*]

[a] International University of Health and Welfare, Department of Social Medical Sciences, 4-3, Kozunomori, Narita-shi, Chiba-ken, 286-8686, Japan
[b] Innovation & Research Support Center, International University of Health and Welfare, 4-1-26 Akasaka, Minato Ward, Tokyo, 107-8402, Japan
[c] Department of Clinical Laboratory, International University of Health and Welfare Narita Hospital, 852 Hatakeda, Narita City, Chiba, 286-8520, Japan
[d] Department of Clinical Laboratory, International University of Health and Welfare Mita Hospital, 1-4-3 Mita, Minato-Ward, Tokyo, 108-8329, Japan
[e] Department of Infectious Diseases, International University of Health and Welfare Narita Hospital, 852 Hatakeda, Narita City, Chiba, 286-8520, Japan
[f] International University of Health and Welfare School of Health Sciences, 4-3, Kozunomori, Narita-shi, Chiba-ken, 286-8686, Japan
[g] Department of Infectious Diseases, International University of Health and Welfare, Chiba, Japan

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ABSTRACT

Introduction: A limited number of studies have shown a decline in antibody titers in healthcare workers beyond six months after the second dose of the BNT162b2 vaccine, and has been insufficiently investigated yet in the respective Asian ethnic groups.
Methods: We conducted a longitudinal observational study on 187 healthcare workers and other personnel and healthy adults at least eight months after vaccination at the International University of Health and Welfare.
Results: The baseline (before the third dose of BNT162b2) anti-receptor binding domain (RBD) IgG level was 569 [377–943] AU/mL 245[240–250] days after the second dose. The mean antibody titer of participants aged 20–29 years was 4.6 times higher than that of participants aged 70–79 years. After booster vaccination, serum anti-RBD antibody levels were elevated in all participants with a median titer of 23,250[14,612–33,401] AU/mL 21 [19–23] days after the third dose. The median post-booster antibody titers in the 20–29, 30–39, 40–49, 50–59, 60–69, and 70–79 years age groups were 30.6, 33.0, 33.8, 27.4, 50.1, and 90.3 times, respectively, higher than the pre-booster ones. Antibody levels were 15% lower in daily drinkers compared to nondrinkers, suggesting that daily alcohol consumption can prevent antibody levels from increasing after vaccination. Our results show decreased antibody titers after two doses of the vaccine, especially in the elderly; however, the third dose of the vaccine resulted in a significant increase in antibody titers in all age groups.
Conclusions: We provided information on antibody responses following primary and booster doses of the BNT162b2 mRNA COVID-19 vaccine in Japan.

1. Introduction

One of the most effective measures for COVID-19 control is vaccination. In countries where the vaccine was authorized for emergency use in December 2020 (earlier than it was authorized in the rest of the world), observational studies have shown that the vaccine is highly effective in preventing both symptomatic and asymptomatic infections [1,2]. Also, the real-world effectiveness of the mRNA booster vaccines against breakthrough infections has been reported in people after two doses of the vaccine, leading to recommendations for a third booster dose [3]. Although evidence of the long-term efficacy of the vaccine and the decrease in anti-spike antibody titers has been accumulating, these aspects have not been sufficiently investigated yet in the respective Asian ethnic groups [4,5]. With the variations in the numbers of cases and deaths in different parts of the world, the approved type of vaccination and local viral circulation also differ, leading to different priorities for vaccine allocation and strategies for infection control [6].

In Japan, currently, three types of COVID-19 vaccine (BNT162b2:

Abbreviations: AU, arbitrary units; IgG, Immunoglobulin G; RBD, receptor binding domain.

* Corresponding author. Department of Infectious Diseases, International University of Health and Welfare. 4-3, Kozunomori, Narita-shi, Chiba-ken, 286-8686, Japan.
E-mail address: tetsuya.m@iuhw.ac.jp (T. Matsumoto).

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Pfizer-BioNTech, and mRNA-1273: Moderna, ChAdOx1 nCoV-19: AstraZeneca) have been approved and the national vaccination rate is above 80%. While BNT162b2 was approved in February 2021 and the initial vaccine series in healthcare workers was administered in March 2021, the administration of the third dose of the vaccine for healthcare workers started in December 2021. To our knowledge, a limited number of unpublished studies have shown a decline in antibody titers in healthcare workers beyond six months after the second dose of the vaccine [5]. Even data on immunological waning after primary series and analytic data on additional vaccinations are scarce in Japan [7]. Therefore, we evaluated the immunogenicity of the vaccine for healthcare workers in consideration of the spread of the sixth wave of the infection caused by the appearance of the omicron variant. This is a real-world longitudinal survey that aimed to assess the immune responses of the boosters in Japanese healthcare workers eight months after the second dose of the vaccine.

2. Materials and methods

2.1. Study design and population

We conducted a longitudinal observational study involving healthcare workers, other personnel, and healthy adults at least eight months after the second dose of the vaccine at the International University of Health and Welfare. The study protocols were approved by the ethics committee of the International University of Health and Welfare. (21-Nr-067). Written informed consent was obtained from all participants. We recruited 187 participants and drew peripheral blood samples from them to measure the titers of SARS-CoV-2 anti-receptor binding domain IgG antibodies before the third dose of BNT163b2 (Pfizer-BioNTech) and 2–3 weeks after the booster doses. They were notified of their anti-S antibody test results after each site visit.

From November 2021 to the end of December 2021, we enrolled people aged 18 years or older, people with no history of suspected clinical SARS-CoV-2 infection before receiving the third dose of the vaccine. Those who had already received the third dose of the vaccine were excluded. The volunteers had received the first dose of the vaccine from March to April of the year 2021. The second dose was administered 21 days after the first one.

The demographic characteristics and comorbidity data were collected after participants submitted their electronic questionnaire responses. The participants were considered eligible for this study if they met the following criteria: age of more than 20 years, healthy adults, took the second dose of the vaccine at least eight months earlier, no past history of COVID-19 infection, and no immunosuppression. The study data were stratified by age group (20–29, 30–39, 40–49, 50–59, 60–69, 70–79 based on the electronic survey), sex, and occupations.

2.2. Serology assays

Serology assays were conducted via the chemiluminescence microparticle immunoassay (CMIA) technique using SARS-CoV-2 IgG II Quant for the quantitative determination of IgG antibodies to SARS-CoV-2 (Abbott Laboratories) and the Architect i20002SR immunoassay analyzer (Abbott Laboratories) according to the manufacturer's instructions. IgG antibody titers >50 AU/mL (as the cut-off set by the manufacturer) were considered indicative of seropositivity. Antibody titers against the spike receptor binding domain (RBD) of SARS-CoV-2 were measured at each time-point in all serum samples.

2.3. Statistical analysis

The results of the total anti-SARS-CoV-2 RBD antibody testing were presented as median values and interquartile ranges (IQRs). Univariate relationships between antibody levels and other variables (e.g., age, sex, and the baseline total anti-SARS-CoV-2 RBD antibody level) were assessed using Spearman's correlation coefficient. All statistical analyses were conducted using SAS version 9.4 (SAS Institute Inc.).

2.4. Results

A total of 187 healthcare workers and other personnel in IUHW hospitals were included in this study. The demographics and background information of the study participants are summarized in Table 1. One hundred and eighty-five out of 187 (98.9%) ethnicities were Asian, and there were 134 (71.7%) individuals with BMIs of less than 25.

The median baseline (before the third dose of BNT162b2) anti-RBD IgG level was 569 (median) [377–943(interquartile range (IQR)] AU/mL 245[240–250] days after the second dose of the vaccine. Almost all eligible subjects received the second dose of the vaccine between March and May of the year 2021. Thereafter, the waning of the anti-RBD antibody titers beyond eight months was observed in the third vaccination period, from December 2021 to January 2022. The scatter plot tended to be a negatively correlated line (Fig. 1). The anti-receptor binding domain (RBD) IgG levels after two doses of the vaccine gradually decreased and the mean antibody titer was 1000 AU/mL after 200 days.

After the booster doses of the vaccine, serum anti-SARS-CoV-2S antibody levels were increased in all participants with a median titer of 23,250[14,612–33,401] AU/mL 21[19–23] days after the third vaccination. However, baseline titers were higher among participants aged 30 years or less than in those aged over 70 years. Comparisons of pre- and post-booster vaccination median antibody titers revealed that the post-booster antibody titers in the 20–29, 30–39, 40–49, 50–59, 60–69, 70–79 years age groups were 30.6, 33.0, 33.8, 27.4, 50.1, 90.3 times, respectively, higher than the pre-booster titers (Fig. 2).

Antibody titers were significantly associated with several baseline factors that predict antibody responses. Some baseline factors that predict antibody responses indicated that the BMI had positive correlations and age had negative correlations with antibody responses. IgG antibody kinetics after the third dose of the vaccine and to associate these changes with the demographic characteristics and coexisting conditions of the participants. Antibody levels were 15% lower in daily drinkers than in

Table 1
Baseline demographic and cohort characteristics (n = 187).

Characteristics	No.(%)
Age, Median(IQR)	45 (27, 31–58)
Sex	
Men	93 (49.7)
Women	94 (50.3)
Ethinicity	
Asian	185 (98.9)
Others	2 (1.1)
Occupation	
Healthcareworkers	158 (84.5)
Others	29 (15.5)
BMI (Body Mass Index: kg/m^2)	
<25	134 (71.7)
25–30	46 (24.6)
30<	7 (3.7)
Comorbidities	
Hypertension	36 (19.3)
Diabetes	2 (1.1)
Dyslipidemia	15 (8.0)
Respiratory disease	9 (4.8)
Heart disease	11 (5.9)
Renal disease	2 (1.1)
Liver Disease	2 (1.1)
Immune disorders	1 (0.5)
None	143 (76.5)
Others	14 (7.5)
Smoking habits	
Past	45 (24.1)
Current	17 (9.1)
Never	125 (66.8)

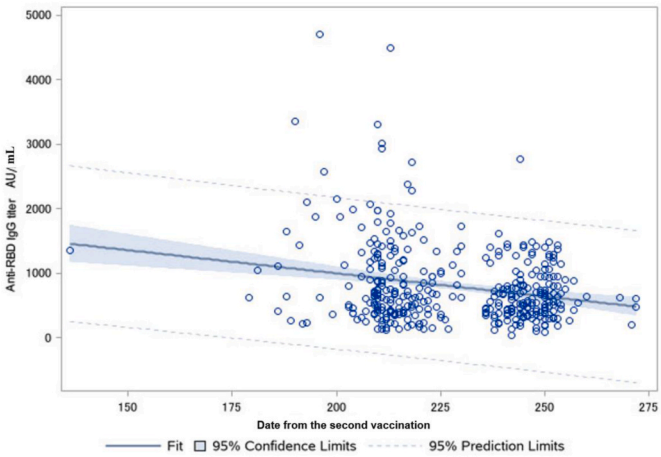

Fig. 1. Timing of the second vaccination and decay of antibody titers. Participants with a history of COVID19 infection and antibody titers in excess of 10,000 were excluded. Abbreviations: AU: arbitrary units, IgG: Immunoglobulin G, RBD: receptor binding domain.

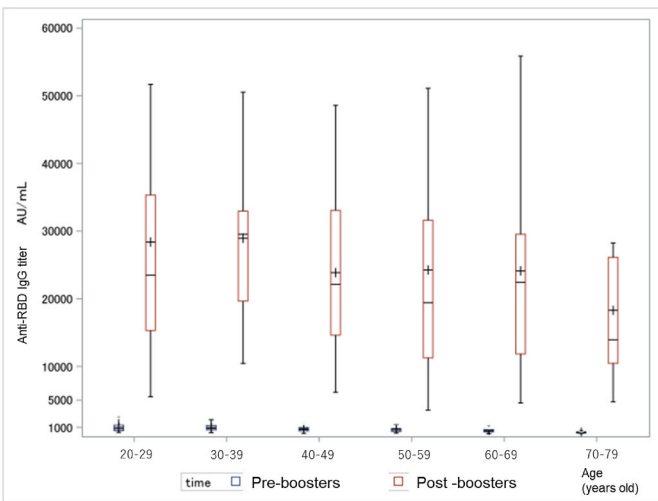

Fig. 2. Distribution of anti-RBD IgG titers according to age before and after the third dose of BNT162b2
The lines in the box indicate the 75th percentile, median, and 25th percentile. The whiskers extend the largest and smallest value by up to 1.5 times the interquartile range from the 75th and 25th percentiles.
Each box-and-whisker diagram represents the association between the increase in antibody titers for the third vaccine according to age.

nondrinkers, suggesting that daily alcohol consumption can prevent antibody levels from increasing after vaccination (Fig. 3). The tendency of smokers to have lower antibody levels than non-smokers was also observed; however, the effect was smaller than that of the participants' drinking habits.

2.5. Discussion

We conducted a longitudinal observational study of the anti-SARS-CoV-2 antibody titers before and after the third dose of the BNT162b2/Pfizer vaccine among Japanese healthcare workers and volunteers. As compared to a previous cohort study of healthcare workers, anti-RBD IgG antibody titers decreased by a similar amount six months after the second dose of the vaccine in our cohort, which also provided mitigation information at eight months [5,8]. IgG kinetics also revealed differences in immunogenicity by age group.

The longevity of the immunity conferred by IgG against COVID-19 waned six months after the second dose of the vaccine and waxed again after nine months. Little is known about the antibody titer threshold for infection; however, there was an increased risk of infection after five months [9]. Antibody titers were significantly lower in participants who were at least 70 years old than in those who were 30 years old or younger during the study period. Based on the Japanese results of this study, the decline in anti-RBD titers following second vaccinations was consistent with the findings of previous studies [3,7,10]. During the end-of-study periods, even though the increase in antibody titers among the ages of 70 was lower than that among youths, all participants became significantly seropositive. Nevertheless, the median antibody titers, which increased pre- and post-booster vaccination, recorded higher values among participants in their 70s than among those who were younger. Our results are consistent with the finding that the booster dose reduced the rate of confirmed infection and severe illness by a similar factor across all age groups from the teens to 80s [11].

Referring to the results of the association of anti-RBD titers after the third dose of the vaccine with baseline predicting factors, lower IgG titers after the boosters were associated with increased age [12,13]and alcohol consumption. In our cohort, participants' drinking habits were the factor that most significantly affected immunogenicity after the third dose. There are two conflicting reports concerning alcohol consumption; it was identified as a negative predictor in one study [14], while the other suggested little associations between the expected increase in antibody titers and participants' drinking habits [15]. To our knowledge, there is a paucity of information on the specific mechanism by which alcohol affects immunogenicity.

This finding differs slightly from the results of previous studies that demonstrate reduced antibody responses among patients on immunosuppressive regimens [16].

Considering our results, we could predict the decline in the immunoglobulin titers of Japanese people who are ready for the third dose of the vaccine. As the world strives for the widespread application of the initial vaccine series, scientific literature on breakthrough infections among fully vaccinated healthcare workers is gradually increasing [17]. The findings that vaccination with three doses of the mRNACOVID-19 vaccine, as compared with two doses, was associated with a reduction in the rate of symptomatic disease for both the Omicron and Delta variants [18,19]. As this pandemic (by the emergence of its variants such as omicron) continues, the determining immune correlates of protection after vaccination become important strategies to prolong host immunity to protect the immunocompromised population against SARS-CoV-2.

Our study has some limitations. First, it was a cohort observational study carried out in Japan that included mostly healthy adults. Moreover, immunosuppression was not identified as an independent predictor because our study population was mostly composed of healthy workers and only 1 (0.5%) individual was immunocompromised. Second, the neutralizing activity was not measured simultaneously. However, the assay we employed has been shown closely associated with the titer of the neutralizing antibody [7], and most of our study subjects achieved a serum antibody level above the optimal cut-off of 10,000 U/mL, which predicts a neutralizing activity of the Omicron variant [20].

In conclusion, we provided information on antibody responses following primary and booster doses of the BNT162b2 mRNA COVID-19 vaccine in Japan.

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Authorship statement

Retsu Fujita, Yasuyuki Kato, Mitsuaki Nagasawa and Tetsuya

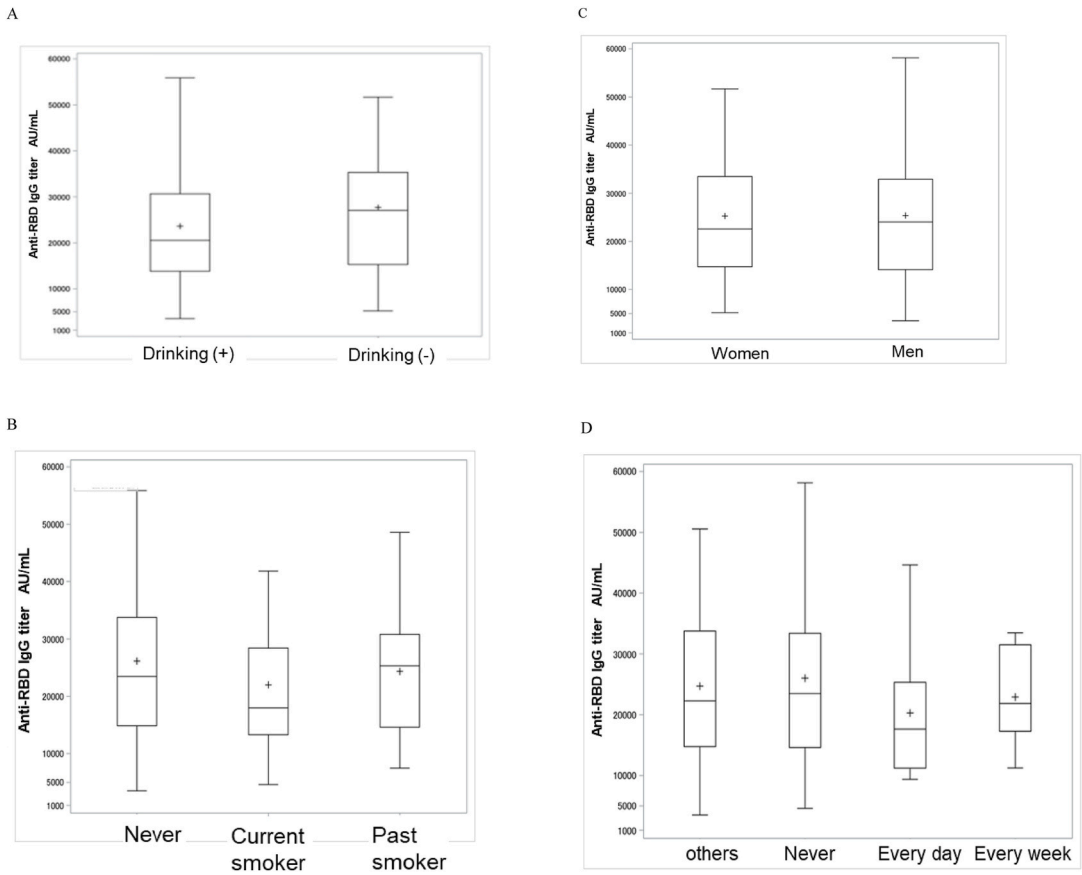

Fig. 3. Distribution of anti-RBD IgG titers after the third dose of BNT162b2 for each predictive factor.
For each boxplot, from top to bottom, the box-and-whisker diagram represents the association between the increase in antibody titers for the third vaccine for each factor.
A: drinking habits, B: smoking habits, C: sex, D: frequency of exposure to COVID-19 patients
Abbreviations, AU: arbitrary units, IgG: Immunoglobulin G, RBD: receptor binding domain.

Matsumoto were responsible for the organization and coordination of the study. Retsu Fujita was the chief investigator and responsible for the data analysis. Miku Tamura, Retsu Fujita, Tomoaki Sato, and Ryohei Sato were contributors of acquisition of data. All authors contributed to the writing of the final manuscript.

Declaration of competing interest

Lecture fee from MSD, Pfizer (T. Matsumoto).
There are no additional relationships or activities to disclose.

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