Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Research Note

Antibody responses to BNT162b2 mRNA COVID-19 vaccine and their predictors among healthcare workers in a tertiary referral hospital in Japan

Takahiro Kageyama 1,1, Kei Ikeda 1,1, Shigeru Tanaka 1,1, Toshibumi Taniguchi 2, Hidetoshi Igari 2,3, Yoshihiro Onouchi 4, Atsushi Kaneda 5, Kazuyuki Matsushita 6, Hideki Hanaoka 7, Taka-Aki Nakada 8, Seiji Ohtori 9, Ichiro Yoshino 10, Hisahiro Matsubara 11, Toshinori Nakayama 12, Koutaro Yokote 13,*, Hiroshi Nakajima 1,3,*

1) Department of Allergy and Clinical Immunology, Graduate School of Medicine, Japan
2) Department of Infectious Diseases, Chiba University Hospital, Japan
3) Chiba University Hospital COVID-19 Vaccine Center, Japan
4) Department of Public Health, Graduate School of Medicine, Japan
5) Department of Molecular Oncology, Graduate School of Medicine, Japan
6) Division of Laboratory Medicine, Chiba University Hospital, Japan
7) Clinical Research Centre, Chiba University Hospital, Japan
8) Department of Emergency and Critical Care Medicine, Graduate School of Medicine, Japan
9) Department of Orthopaedic Surgery, Graduate School of Medicine, Japan
10) Department of General Thoracic Surgery, Graduate School of Medicine, Japan
11) Department of Frontier Surgery, Graduate School of Medicine, Japan
12) Department of Immunology, Graduate School of Medicine, Japan
13) Department of Endocrinology, Hematology and Gerontology, Graduate School of Medicine, Chiba University, Chiba, Japan

abstract

Objectives: This study aimed to determine antibody responses in healthcare workers who receive the BNT162b2 mRNA COVID-19 vaccine and identify factors that predict the response.

Methods: We recruited healthcare workers receiving the BNT162b2 mRNA COVID-19 vaccine at the Chiba University Hospital COVID-19 Vaccine Center. Blood samples were obtained before the 1st dose and after the 2nd dose vaccination, and serum antibody titers were determined using Elecsys® Anti-SARS-CoV-2, an electrochemiluminescence immunoassay. We established a model to identify the baseline factors predicting post-vaccine antibody titers using univariate and multivariate linear regression analyses.

Results: Two thousand fifteen individuals (median age 37-year-old, 64.3% female) were enrolled in this study, of which 10 had a history of COVID-19. Before vaccination, 21 participants (1.1%) had a detectable antibody titer (≥0.4 U/mL) with a median titer of 35.9 U/mL (interquartile range [IQR] 7.8 – 65.7). After vaccination, serum anti-SARS-CoV-2 antibodies (≥0.4 U/mL) were detected in all 1774 participants who received the 2nd dose with a median titer of 2060.0 U/mL (IQR 1250.0 – 2650.0). Immunosuppressive medication (p < 0.001), age (p < 0.001), time from 2nd dose to sample collection (p < 0.001), glucocorticoids (p = 0.020), and drinking alcohol (p = 0.037) were identified as factors predicting lower antibody titers after vaccination, whereas previous COVID-19 (p < 0.001), female (p < 0.001), time between 2 doses (p < 0.001), and medication for allergy (p = 0.024) were identified as factors predicting higher serum antibody titers.

* Corresponding author. Hiroshi Nakajima, Department of Allergy and Clinical Immunology, Graduate School of Medicine, Chiba University, 1-8-1 Inohana, Chiba City, 260-8670, Japan. Fax: +81 43 226 2199.
** Corresponding author. Koutaro Yokote, Department of Endocrinology, Hematology and Gerontology, Graduate School of Medicine, Chiba University, 1-8-1 Inohana, Chiba City, 260-8670, Japan.
E-mail addresses: kyokote@faculty.chiba-u.jp (K. Yokote), nakajimh@faculty.chiba-u.jp (H. Nakajima).
1 These authors equally contributed to this work.
Our data demonstrate that healthcare workers universally have good antibody responses to the BNT162b2 mRNA COVID-19 vaccine. The predictive factors identified in our study may help optimize the vaccination strategy. Takahiro Kageyama, Clin Microbiol Infect 2021;27:1861.e1–1861.e5 © 2021 European Society of Clinical Microbiology and Infectious Diseases. Published by Elsevier Ltd. All rights reserved.

Introduction

BNT162b2 mRNA vaccine against COVID-19 has shown promising efficacy both in a clinical trial [1] and in nationwide mass vaccination settings [2]. The vaccine has also shown short-term efficacy in a large-scale prospective cohort study targeting healthcare workers, a population that should be prioritized for vaccination [3]; however, the factors that predict the effectiveness of BNT162b2 mRNA vaccine have not been fully explored.

As the humoral responses have been shown to play essential roles in the protection against and the survival from SARS-CoV-2 infection [4–6], the antibody status after vaccination can provide important information to predict long-term effectiveness and to optimize the vaccination strategy. However, antibody responses after vaccination have been assessed only in small-scale studies [7–12]. Here, we report the antibody responses and their predictive factors in 2015 healthcare workers who received the BNT162b2 mRNA COVID-19 vaccine.

Methods

We recruited healthcare workers in Chiba University Hospital who were receiving the BNT162b2 mRNA COVID-19 vaccine (Pfizer, Inc., and BioNTech) at the Chiba University Hospital COVID-19 Vaccine Center.

Background information was collected by a web-based questionnaire. We considered that a subject had a history of COVID-19 when 1) the subject answered so in the web questionnaire and 2) the subject had been registered as a PCR-positive case in the hospital registry.

Blood samples were obtained 0–2 weeks before the 1st dose and 2–5 weeks after the 2nd dose of vaccination. Antibody responses were analyzed using Elecsys® Anti-SARS-CoV-2 on Cobas 8000 e801 module (Roche Diagnostics, Rotkreuz, Switzerland). This system allows for the quantitative detection of antibodies, predominantly IgG, aiming at the SARS-CoV-2 spike protein receptor-binding domain. The measurement threshold is ≥0.4 U/mL and values ≥0.8 U/mL are considered positive. Samples with a titer >250 U/mL were diluted x10 at a time until the titer became ≤250 U/mL according to the manufacturer’s protocol.

We first performed univariate linear regression analyses to identify factors associated with the serum anti-SARS-CoV-2 antibody titer after vaccination. We next performed a multivariate linear regression analysis with a stepwise method using factors that showed a p-value <0.1 in univariate analyses. Statistical analyses were performed using SPSS version 23.0 (IBM, Armonk, NY). A two-sided p-value <0.05 was considered statistically significant.

The study procedures for sample collection and those for analyses were approved by Chiba University Ethics Committee on February 24th, 2021 (No. HS202101-03) and April 21st, 2021 (No. HS202104-01), respectively. All study subjects gave written informed consent before undergoing any study procedures.

Results

Out of 2838 employees in Chiba University Hospital, 2549 (89.8%) received at least one dose of BNT162b2 mRNA COVID-19 vaccine (30 μg) from March 3rd to April 9th, 2021, and 2015 individuals (71.0%) were enrolled in this study. Demographics and background information are summarized in Table 1.

Before vaccination, serum anti-SARS-CoV-2S antibody (≥0.4 U/mL) was detected only in 21 subjects (1.1%) with a median titer of 35.9 U/mL (IQR 7.8–65.7). Eighteen subjects (0.9%) had a positive titer (≥0.8 U/mL) and 8 out of these 18 subjects (44.4%) had a history of COVID-19.

After vaccination, serum anti-SARS-CoV-2S antibody (≥0.4 U/mL) was detected in all 1774 participants who received the 2nd dose with a median titer of 2060.0 U/mL (IQR 1250.0–2650.0) (Supplementary Fig. 1A). Only one subject, who had received aggressive immunosuppressive treatment for a severe autoimmune condition, had a negative titer (0.7 U/mL). The distribution of post-vaccination antibody titers according to age and sex is shown in Supplementary Fig. 1B. In those who were seropositive before vaccination, the antibody titers substantially increased with a median fold change of 412.4 (IQR 309.2–760.5) following the 2nd dose.

The results of univariate and multivariate linear regression analyses are summarized in Table 1. The factors retained in the final multivariate model (adjusted R² 0.188) were immunosuppressive medication, age, time from 2nd dose to sample collection, previous COVID-19, sex, time between 2 doses, glucocorticoids, medication for allergy, and drinking alcohol (Fig. 1).

Discussion

All subjects who received 2 doses of BNT162b2 mRNA COVID-19 vaccine had a detectable level of serum anti-SARS-CoV-2S antibody, and all but one subject who were seronegative before vaccination became seropositive (99.9%). In addition, all of 18 subjects who were already seropositive before vaccination showed substantial antibody responses after the 2nd dose. These results are consistent with previous smaller-scale studies [7,8,11,12] and indicate that the vast majority of young-adult healthcare workers have good antibody responses following 2 doses of the BNT162b2 vaccine.

The large sample size of our study allowed for establishing a stable multivariate model to determine background factors that predict antibody responses. The strongest and the most significant factor was receiving immunosuppressive drugs. Receiving glucocorticoids was also identified as an independent predictor even though our study population was mostly healthy workers and only 14 (0.9%) and 9 (0.6%) were taking glucocorticoids and immunosuppressant, respectively. Our data confirm the results of previous studies which demonstrated reduced antibody responses among patients on immunosuppressive regimens [13].

Unexpectedly, medication for allergy was also identified as a factor significantly associated with higher antibody titers. Although we have no information on the drug and diagnosis for the medication, we speculate that the majority were taking anti-histamine drugs for cedar pollen allergy, which is very common in Japan in spring. Interestingly, some studies have suggested potential therapeutic effects of histamine H1 receptor antagonists on COVID-19 [14]. Together with alcohol consumption as a negative predictor, these novel associations deserve further investigation.
Table 1
Background information and results of univariate/multivariate linear regression analysis for post-vaccine antibody titer

| Variable | All (n = 2015) | Post-vaccine antibody titer available (n = 1774) | Linear regression analysis for post-vaccine antibody titer |
|----------|----------------|-----------------------------------------------|----------------------------------------------------------|
|          | Data available, n | Value | Data available, n | Value | Univariate | Multivariate |
|          |                 |        |                 |       | Regression coefficients (B) | 95% confidence interval | Regression coefficients (B) | 95% confidence interval |
| Age (year-old), median (IQR) | 2015 | 37 (29-47) | 1774 | 39.3 | -0.016 | -0.020 -- -0.011 | -0.016 | -0.021 -- -0.012 |
| Sex female, n (%) | 2015 | 1296 (64.3) | 1774 | 1168 (65.8) | 0.306 | 0.200 -- 0.412 | 0.264 | 0.156 -- 0.372 |
| Nationality Japanese, n (%) | 2015 | 2004 (99.5) | 1774 | 1769 (99.5) | 0.303 | -0.412 -- 1.018 |
| Job category | 2015 | 1774 | | | 0.211* | | 0.102 -- 0.320 |
| Nurse | | 672 (33.3) | 559 (31.5) | | | | |
| Doctor | | 589 (29.2) | 494 (27.8) | | | | |
| Pharmacist | | 58 (2.9) | 57 (3.2) | | | | |
| Dentist | | 19 (0.9) | 11 (0.6) | | | | |
| Others | | 677 (33.6) | 653 (36.8) | | | | |
| Body mass index category | 1515 | 1512 | | | 0.014 | | -0.097 -- 0.124 |
| <18.5 | | 150 (9.9) | 150 (9.9) | | | | |
| 18.5-25 | | 1120 (73.9) | 1118 (73.9) | | | | |
| ≥25 | | 245 (16.2) | 244 (16.1) | | | | |
| Smoking | 1515 | 1512 | | | -0.200 | | -0.308 -- -0.093 |
| Never, n (%) | | 1141 (75.3) | 1139 (75.3) | | | | |
| Ex-smoker, n (%) | | 323 (21.3) | 323 (21.4) | | | | |
| Current smoker, n (%) | | 51 (3.4) | 50 (3.3) | | | | |
| Alcohol | 1515 | 1512 | | | -0.111 | | -0.197 -- -0.024 | -0.084 | -0.163 -- -0.005 |
| No, n (%) | | 417 (27.5) | 417 (27.6) | | | | |
| Sometimes, n (%) | | 863 (57.0) | 860 (56.9) | | | | |
| Every day, n (%) | | 235 (15.5) | 235 (15.5) | | | | |
| Comorbidities | 1515 | 1512 | | | | | |
| Asthma, n (%) | | 158 (10.4) | 158 (10.4) | | -0.041 | -0.224 -- -0.141 |
| Atopic dermatitis, n (%) | | 134 (8.8) | 134 (8.9) | | -0.007 | -0.204 -- -0.189 |
| Hypertension, n (%) | | 114 (7.5) | 114 (7.5) | | -0.343 | -0.553 -- -0.132 |
| Dyslipidemia, n (%) | | 97 (6.4) | 97 (6.4) | | -0.213 | -0.441 -- -0.014 |
| Thyroid disease, n (%) | | 55 (3.6) | 55 (3.6) | | 0.092 | -0.206 -- 0.390 |
| Malignancy, n (%) | | 36 (2.4) | 36 (2.4) | | -0.022 | -0.388 -- -0.344 |
| Diabetes mellitus, n (%) | | 25 (1.7) | 25 (1.7) | | -0.388 | -0.826 -- 0.049 |
| Autoimmune disease, n (%) | | 13 (0.9) | 13 (0.9) | | -2.609 | -3.812 -- -2.019 |
| Ischemic heart disease, n (%) | | 5 (0.3) | 5 (0.3) | | -0.485 | -1.458 -- 0.487 |
| Cerebral infarction, n (%) | | 4 (0.3) | 4 (0.3) | | 0.179 | -0.909 -- 1.266 |
| Interstitial lung disease, n (%) | | 2 (0.1) | 2 (0.1) | | -5.383 | -8.895 -- -1.870 |
| Chronic obstructive pulmonary disease, n (%) | | 0 (0.0) | 0 (0.0) | | NA | NA |
| Current medication | 1515 | 1512 | | | | | |
| Allergy, n (%) | | 188 (12.4) | 188 (12.4) | | 0.150 | -0.019 -- 0.319 | 0.177 | 0.023 -- 0.331 |
| Hypertension, n (%) | | 102 (6.7) | 102 (6.7) | | -0.397 | -0.619 -- -0.176 |
| Dyslipidemia, n (%) | | 77 (5.1) | 77 (5.1) | | -0.117 | -0.371 -- 0.137 |
| Inhaled corticosteroid, n (%) | | 32 (2.1) | 32 (2.1) | | -0.300 | -0.687 -- 0.088 |
| Thyroid disease, n (%) | | 27 (1.8) | 27 (1.8) | | -0.027 | -0.449 -- 0.395 |
| Diabetes mellitus, n (%) | | 20 (1.3) | 20 (1.3) | | -0.179 | -0.668 -- 0.309 |
| Glucocorticoids, n (%) | | 14 (0.9) | 14 (0.9) | | -3.366 | -2.294 -- -2.438 |
| Immunosuppressant, n (%) | | 9 (0.6) | 9 (0.6) | | -4.294 | -4.987 -- -3.601 |

(continued on next page)
While only 10 participants (0.5%) in our study had a history of COVID-19, it was the fourth most significant factor in our multivariate model. Its influence might have been underestimated since 2 participants who had the highest titers did not have a history of previous COVID-19 but both were seropositive before vaccination, and one had had close contact with an infected individual. Again, this result is consistent with previous reports [11,12,15,16] and consolidates the evidence that the BNT162b2 vaccine induces more robust antibody responses in individuals previously infected with SARS-CoV-2.

Among demographic factors, older age has been repeatedly reported to associate with reduced antibody responses after COVID-19 vaccination [7,8,11,12]. Our study population is younger than those in previous studies and supplement the evidence. Sex difference has also been reported to associate with antibody responses to various degrees [8,11,12]. Our large-scale data confirm the notion that women tend to have a greater antibody response to the BNT162b2 vaccine than men.

Our study has some limitations. First, this is a single-center study in Japan with mostly Japanese subjects. Second, neutralizing activity was not measured. However, the assay we employed has been shown closely correlated with the titer of neutralizing antibody [17], and 99.5% of our study subjects achieved a serum antibody level above the cut-off of 133 U/mL to predict a neutralizing activity. Third, we only assessed the antibody responses and did not assess the cellular ones. Fourth, most clinical information was collected with a questionnaire and cannot be verified.

Nevertheless, we provide large-scale data on antibody responses to the BNT162b2 mRNA COVID-19 vaccine. Universally good responses demonstrated in our study further support the use of this vaccine in a wide range of populations, and the predictive factors identified may help optimize the vaccination strategy and generate hypotheses for future studies.

**Transparency declaration**

We have no conflict of interest to declare. This study was supported by a donation to Chiba University Hospital and the Future Medicine Founds at Chiba University.

---

**Table 1** (continued)

| Variable                                                                 | All (n = 2015) | Post-vaccine antibody titer available (n = 1774) |
|--------------------------------------------------------------------------|---------------|-----------------------------------------------|
| **Linear regression analysis for post-vaccine antibody titer**           |               |                                               |
| Data available, n. Value                                                |               |                                               |
| Linear regression analysis for post-vaccine antibody titer               |               |                                               |
| Data available, n. Value                                                |               |                                               |
| Insulin, n (%)                                                          | 3 (0.2)       | 3 (0.2)                                      |
| Antimicrobial, n (%)                                                     | 3 (0.2)       | 3 (0.2)                                      |
| Previous COVID-19, n (%)                                                | 10 (0.5)      | 9 (0.5)                                      |
| Flu symptoms within a year, n (%)                                       | 1515          | 1512                                         |
| Exposure to COVID-19, n (%)                                             | 1515          | 1512                                         |
| Hardly, n (%)                                                           | 1333 (88.0)   | 1331 (88.0)                                 |
| Time between 1st and 2nd doses (day), median (IQR)                      | 1574          | 1574                                         |
| Time between 2nd dose and sample collection (day), median (IQR)         | 14 (14-21)    | 14 (14-21)                                  |

**Fig. 1.** Multivariate linear regression model to predict anti-SARS-CoV-2S antibody titers after vaccination. Shown are the variables retained in the final multivariate linear regression model to explain anti-SARS-CoV-2S antibody titers after vaccination. A dot and bar represent standardized coefficient β and 95% confidence interval for the variable.
Author's contributions

Conception of the work: TK, KI, ST, HI, TN, KY, HN. Data collection: TK, KI, ST, TT, HI, YO, AK, KM, HH, TAN, SO, IY, HM. Data analysis and interpretation: TK, KI, ST, HI, KY, HN. Drafting of the article: TK, KI, ST, HN. Critical revision of the article: TK, KI, ST, HN. Final approval of the version to be published: TK, KI, ST, TT, HI, YO, AK, KM, HH, TAN, SO, IY, HM, TN, KY, HN.

Acknowledgements

We thank all staff in Chiba University Hospital for supporting sample collection.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2021.07.042.

References

[1] Polack FP, Thomas SJ, Kitchin N, Absalon J,urtman A, Lockhart S, et al. Safety and efficacy of the BNT162b2 mRNA covid-19 vaccine. N Engl J Med 2020;383:2603–15.
[2] Dagan N, Bara N, Kepten E, Miron O, Perchik S, Katz MA, et al. BNT162b2 mRNA covid-19 vaccine in a nationwide mass vaccination setting. N Engl J Med 2021;384:1412–23.
[3] Hall VJ, Foulkes S, Saei A, Andrews N, Oguti B, Charlett A, et al. COVID-19 vaccine coverage in health-care workers in England and effectiveness of BNT162b2 mRNA vaccine against infection (SIREN): a prospective, multicentre, cohort study. Lancet 2021;397:1725–35.
[4] Dispinseri S, Secchi M, Pirillo MF, Tolazzi M, Borghi M, Brigatti C, et al. Neutralizing antibody responses to SARS-CoV-2 in symptomatic COVID-19 is persistent and critical for survival. Nat Commun 2021;12:2670.
[5] Lumley SF, O’Donnell D, Stoesser NE, Matthews PC, Howarth A, Hatch SB, et al. Antibody status and incidence of SARS-CoV-2 infection in health care workers. N Engl J Med 2021;384:333–40.
[6] Hall VJ, Foulkes S, Charlett A, Atti A, Monk EJM, Simmons R, et al. SARS-CoV-2 infection rates of antibody-positive compared with antibody-negative health-care workers in England: a large, multicentre, prospective cohort study (SIREN). Lancet 2021;397:1459–69.
[7] Muller L, Andrei M, Mokosz W, Drexler I, Walotka L, Grothmann R, et al. Age-dependent immune response to the Biontech/Pfizer BNT162b2 COVID-19 vaccination. Clin Infect Dis 2021. https://doi.org/10.1093/cid/ciaa381, ciaa381.
[8] Terpou E, Troupakos IP, Apostolakou F, Charitaki I, Skirou AD, Marriano N, et al. Age-dependent and gender-dependent antibody responses against SARS-CoV-2 in health workers and octogenarians after vaccination with the BNT162b2 mRNA vaccine. Am J Hematol 2021;96:E257–9.
[9] Favresse J, Bayart JL, Mullier F, Dogne JM, Closset M, Douxfils J. Early antibody response in health-care professionals after two doses of SARS-CoV-2 mRNA vaccine (BNT162b2). Clin Microbiol Infect 2021;51:198.
[10] Walsh E, Frenck Jr RW, Falsey AR, Kitchin N, Gurtman A, et al. Safety and immunogenicity of two RNA-based covid-19 vaccine candidates. N Engl J Med 2020;383:2439–50.
[11] Bayart JL, Morimont L, Closset M, Weers G, Ger Roy T, Gerin V, et al. Confounding factors influencing the kinetics and magnitude of serological response following administration of BNT162b2. Microorganisms 2021;9:1340.
[12] Salvagni GL, Henry BM, di Piazza G, Pighi L, De Notto S, Bragantini D, et al. Anti-SARS-CoV-2 receptor-binding domain total antibodies response in seropositive and seronegative healthcare workers undergoing COVID-19 mRNA BNT162b2 vaccination. Diagnostics (Basel) 2021;11:832.
[13] Boyarsky BJ, Werbel WA, Avery BK, Tobian AAR, Massie AB, Segev DR, et al. Immunogenicity of a single dose of SARS-CoV-2 messenger RNA vaccine in solid organ transplant recipients. JAMA 2021;325:1784–6.
[14] Ge S, Wang X, Hou Y, Lv Y, Wang C, He H. Repositioning of histamine H1 receptor antagonist: doxepin inhibits viropexis of SARS-CoV-2 Spike pseudovirus by blocking ACE2. Eur J Pharmacol 2021;896:173897.
[15] Manisty C, Otter AD, Treibel TA, McKnight J, C019 A, Altmann DM, Brooks T, et al. Antibody response to BNT162b2 in previously SARS-CoV-2-infected individuals. Lancet 2021;397:1057–8.
[16] Ehinger JE, Fert-Boher J, Prinslov L, Wu M, Sun N, Frostkoc J, et al. Antibody responses to the BNT162b2 mRNA vaccine in individuals previously infected with SARS-CoV-2. Nat Med 2021;27:981–4.
[17] Resman Rus K, Korva M, Knap N, Avsic Zupanc T, Poljak M. Performance of the rapid high-throughput automated electrochemiluminescence immunoassay targeting total antibodies to the SARS-CoV-2 spike protein receptor binding domain in comparison to the neutralization assay. J Clin Virol 2021;139:104820.