〈Report〉

Qualitative antibody responses, changes in inflammatory biomarker levels, and adverse reactions following vaccination with the BNT162b2 COVID-19 mRNA vaccine

Hidenori Onishi[1], Osamu Yamamura[1,*], Ippei Sakamaki[2] and Hiromichi Iwasaki[3]

Summary This report describes the antibody response, inflammatory response, and adverse reactions in two men who received the BNT162b2 COVID-19 mRNA vaccine (Pfizer-BioNTech). In Case 1, involving a man in his 50s, immunoglobulin G (IgG) antibodies were detected after the first dose of the vaccine. In contrast, immunoglobulin M (IgM) antibodies were not detected during the observation period. The peak C-reactive protein (CRP) level was 1.31 mg/dL. In Case 2, involving a man in his 40s, both IgG and IgM antibodies were detected after the first dose of the vaccine. The peak CRP level was 2.01 mg/dL. After the second dose, the patient's blood pressure increased to 168/110 mm Hg. The elevated CRP level after vaccination might have been associated with systemic reactions. Furthermore, because one of the patients developed stage III hypertension, post-vaccination monitoring and control of blood pressure should be a requirement.

Key words: COVID-19, BNT162b2 vaccine, Hypertension, Qualitative antibody test, Inflammatory response

1. Introduction

The Pfizer-BioNTech COVID-19 mRNA vaccine, BNT162b2, is one of the most effective measures to protect people from becoming infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and becoming severely ill. While it is highly effective, many adverse reactions have been reported.[1-4] To end this pandemic as soon as possible, the acquisition of immunity (antibodies) through vaccination is essential.

BNT162b2 vaccine delivers genetic information

[1] Department of Community Medicine, Faculty of Medical Sciences, University of Fukui, 23-3 Matsuokashimoaizuki, Yoshida-gun, Eiheiji-cho, Fukui 910-1104, Japan.
[2] Department of Infectious Diseases, Faculty of Medical Sciences, University of Fukui, 23-3 Matsuokashimoaizuki, Yoshida-gun, Eiheiji-cho, Fukui 910-1104, Japan.
[3] Division of Infection and Clinical Immunology, University of Fukui Hospital, 23-3 Matsuokashimoaizuki Yoshida-gun, Eiheiji-cho, Fukui 910-1104, Japan.

*Corresponding author: Osamu Yamamura, Department of Community Medicine, Faculty of Medical Sciences, University of Fukui, 23-3 Matsuokashimoaizuki, Yoshida-gun, Eiheiji-cho, Fukui 910-1104, Japan.
Tel: +81-776-61-8264
Fax: +81-776-61-8270
E-mail: kapi@u-fukui.ac.jp

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(mRNA), which designs glycoproteins called spike (S) proteins on the surface of SARS-CoV-2, and the vaccine induces both production of antibodies against the spike protein and T cell-mediated immunity.[1-3,5] IgM antibody titers against SARS-CoV-2 in the serum of Japanese COVID-19 patients tended to rise about nine days after symptom onset, peak after approximately 15–20 days, and then begin to decrease.[6] Because the IgM antibody persistence period is shorter than that of the IgG antibody, IgM antibody response provides early-stage defense during infections by vaccination, whereas IgG antibody response provides long-term immunity. In addition, IgG antibody titers against spike protein correlate with neutralizing antibody titers, providing us indirect information on the acquisition of functional immunity.[7] Recently, the IgA antibody has also attracted attention, as its production is faster than that of IgM and IgG antibodies, and a correlation with disease severity has been reported.[8]

In a study of adverse reactions after BNT162b2 vaccination in Japanese individuals, adverse reactions occurred in 757 of 983 individuals (77.0%) and 715 of 798 individuals (90.0%), after the first and second doses, respectively.[4] Pain at the injection site (56.0%), muscle pain (42.0%), and fatigue (25.5%) were the most common adverse reactions in the first dose. In the second dose, fatigue (68.0%), pain at the injection site (67.2%), and body temperature above 38 °C (55.0%) were the most common adverse reactions.[4] The number of individuals with a temperature above 37.5 °C increased from 3.0% after the first dose to 44.9% after the second dose ($p < 0.001$, odds ratio: 26.77, 95% confidence interval: 18.03-39.73).[4] Some of the adverse reactions could be explained by inflammation induced by the reaction of immune cells. Tumor necrosis factor-α (TNF-α), a proinflammatory cytokine, was also used as a biomarker in a study involving systemic reactions to the BNT162b2 vaccine.[9]

This report describes the antibody responses and levels of C-reactive protein (CRP), which has been used as a common inflammation marker, in two men during a 30-day observation period after BNT162b2 vaccination, along with details of their adverse reactions.

2. Materials and Methods

Methods

The cost of reagents and antibody test kits was taken into account in the selection of our study cases, which was limited to two participants who could perform the tests for one month, and the first two participants were selected. Both participants provided written informed consent. In this prospective study, blood samples were collected every 24 hours for one month from the first vaccination date, and adverse reactions were monitored. Antibody development after vaccination was confirmed using a qualitative antibody test kit. Body temperature and blood pressure were also measured every 24 hours after vaccination. To measure antibodies and CRP levels, whole blood was collected from the fingertips by self-administration, and the required amount was applied to the test kits according to the respective measurement instructions. Blood pressure was measured using an upper arm sphygmomanometer, twice at each time point.

Equipment and test kits

Antibodies were tested using the Driven-Flow COVID-19 IgG/IgM Antibody Test (ALFA SCIENTIFIC DESIGNS; California, USA): a rapid qualitative antibody test kit based on immunochromatography. The sensitivity and specificity of this test kit are as accurate as being reported: sensitivity of 100% and specificity of 93.8% for IgM antibody; sensitivity of 80.0% and specificity of 100% for IgG; sensitivity of 100% and specificity of 93.8% for the combined antibodies.[10] CRP level was measured using cobas b 101 devices (Roche Diagnostics K.K.; Tokyo, Japan), a simple hematology analyzer based on the latex agglutination method, and cobas b 101 CRP discs (Roche Diagnostics K.K.; Tokyo, Japan). C230 electronic thermometers (Terumo; Tokyo, Japan) and HEM-1021 sphygmomanometers (Omron; Kyoto, Japan) were used to measure body temperature and blood pressure, respectively.

Background of Case 1

Case 1 involved a 52-year-old man who was a non-smoker and did not consume alcohol. He

received two doses of the BNT162b2 vaccine, which were administered intramuscularly into the deltoid muscle, 21 days apart. He had a history of hypertension and diabetes mellitus and had one kidney removed due to an injury sustained in a motor vehicle accident, with no decline in renal function.

Background of Case 2

Case 2 involved a 42-year-old man who was a non-smoker and did not consume alcohol. He received two doses of the BNT162b2 vaccine, which were administered intramuscularly into the deltoid muscle, 21 days apart. The participant had a history of childhood asthma, allergic rhinitis, gastritis, and a benign liver tumor.

3. Results

Progress of Case 1

The IgG antibody response was weakly positive on Day 21 after the first dose, whereas there were no IgM antibodies detected during the 30-day study period (Fig. 1). The patient's body temperature was at the upper limit of normal (37.2 °C) on Day 22, the day after the second dose (Fig. 2). His CRP level was 0.35 mg/dL on Day 2 after the first dose, and 0.36, 1.26, 1.31, 0.87, and 0.42 mg/dL on Days 21 (five hours after the second dose), 22, 23, 24, and 25, respectively; his CRP level was the highest on Day 23, two days after receiving the second dose of the vaccine (Fig. 3). The participant experienced pain at the injection site from Days 0 to 2 after the first dose, and again on the day of the second dose and the day after. After the second dose, he also experienced itching and a rash, which persisted for approximately one week (Table 1). No information on blood pressure for the patient in Case 1 is available.

Progress of Case 2

The IgG and IgM antibodies were weakly positive on Days 16 and 13, respectively (Fig. 1). The participant's body temperature increased to 37.6 °C on Day 22, the day after receiving the second dose of the vaccine (Fig. 2). His CRP levels were 0.45, 0.42, 1.19, 2.01, 0.94, and 0.42 mg/dL on Days 1, 2, 22, 23, 24, and 25, respectively; his CRP level was the highest on Day 23, two days after receiving the second dose of the vaccine (Fig. 3). Adverse reactions included pain at the injection site, chills, and difficulty in raising the arm, which persisted from Days 0 to 3 after the first dose (Table 1). After the

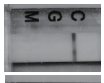
Case 1				Case 2			
Days	Picture	IgM	IgG	Days	Picture	IgM	IgG
Day 0		—	—	Day 0		—	—
Day 7		—	—	Day 7		—	—
Day 14		—	—	Day 13		±†	—
Day 21		—	±†	Day 16		±†	±†
Day 26		—	+	Day 21		±	+
Day 28		—	+	Day 28		+	+
Day 30		—	+	Day 30		+	+

Fig. 1. SARS-CoV-2 immunoglobulin M and immunoglobulin G antibody responses following vaccination with the BNT162b2 COVID-19 mRNA vaccine. IgG, immunoglobulin G; IgM, immunoglobulin M. †A line could be seen with the naked eye but is not clearly visible in the photograph.

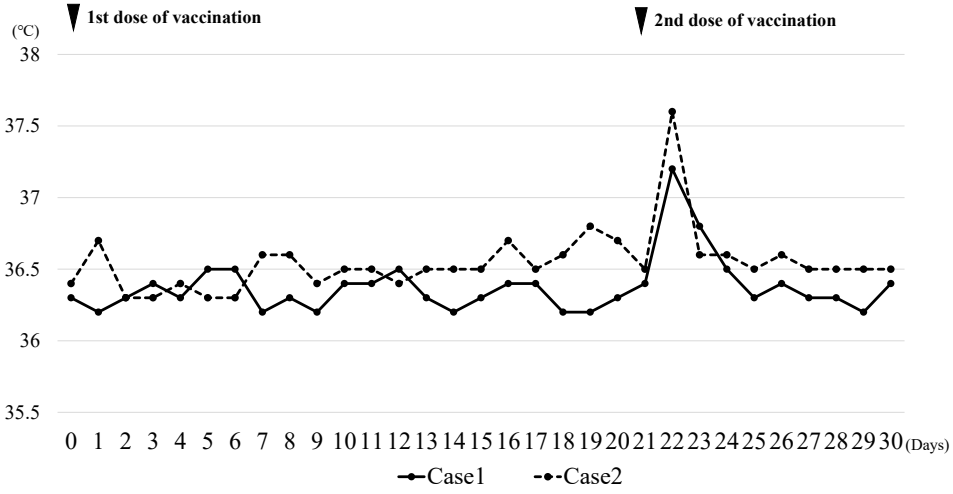


Fig. 2. Time course of change after vaccination (BNT162b2) — Body temperatures in case 1 and case 2.

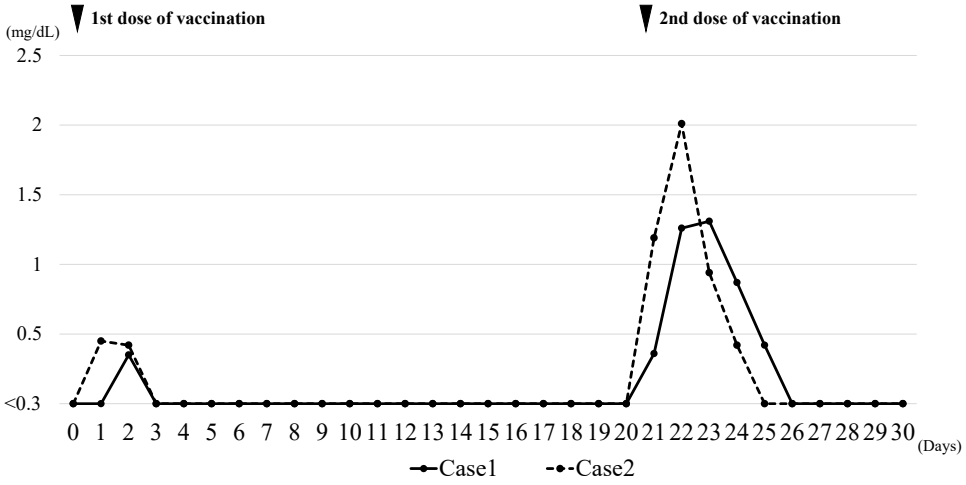


Fig. 3. Time course of change after vaccination (BNT162b2) — CRP in case 1 and case 2.

second dose on Day 21, the participant experienced pain at the injection site, fever, backache, headache, fatigue, and appeared pale (Table 1). He experienced discomfort at the injection site from Days 21 to 27 (Table 1). Because the participant appeared pale and fatigued, his blood pressure was measured several times, and was found to be elevated: 168/110, 140/84, and 141/92 mm Hg on Days 23, 24, and 25, respectively (Fig. 4). His blood pressure had been normal (128/84 mmHg) during a routine medical examination on Day 19 (Fig. 4).

4. Discussion

Since it was not possible to make comparisons between the two cases due to the different backgrounds of the patients, we have reported on each case separately and compared them to previously reported cases. The IgG antibody tests in both our patients in Case 1 and 2 showed very weak positive results on Days 21 and 16, respectively. This suggests that a single vaccination can promote the production of IgG antibodies at approximately the same rate as in individuals who have been previously infected with SARS-CoV-2. In contrast, the IgM antibody test in the patient in Case 2 showed a positive result on Day 13 (two weeks after the first dose), but the patient in Case 1 did not develop a positive IgM result during the observation period. In a cross-sectional study of antibody titers after BNT162b2 vaccination in Japanese subjects, after the first dose, 35% of 23 participants tested positive for IgG antibodies (median: 19 days; interquartile range [IQR]: 17–20 days), and none of them tested

Table 1 Time course of change after vaccination (BNT162b2)—Side effect information

		Case 1	Case 2
Days	Vaccine	Side effects	Side effects
Day 0	1st dose	Pain at the injection site	Pain at the injection site, chills
Day 1		Pain at the injection site	Pain at the injection site, chills, difficulty in raising the arm
Day 2		Pain at the injection site	Pain at the injection site
Day 3		—	Pain at the injection site
Day 21	2nd dose	Pain at the injection site	Pain at the injection site
Day 22		Itching, rash	Pain at the injection site: myalgia, fatigue, fever
Day 23		Itching, rash	Pain at the injection site: backache, fatigue, headache, pallor
Day 24		Itching, rash	Pain at the injection site, fatigue
Day 25		Itching, rash	Discomfort at the injection site
Day 26		Itching, rash	Discomfort at the injection site
Day 27		Itching, rash	Discomfort at the injection site
Day 28		Itching, rash	—
Day 29		Itching, rash	—
Day 30		Itching, rash	—

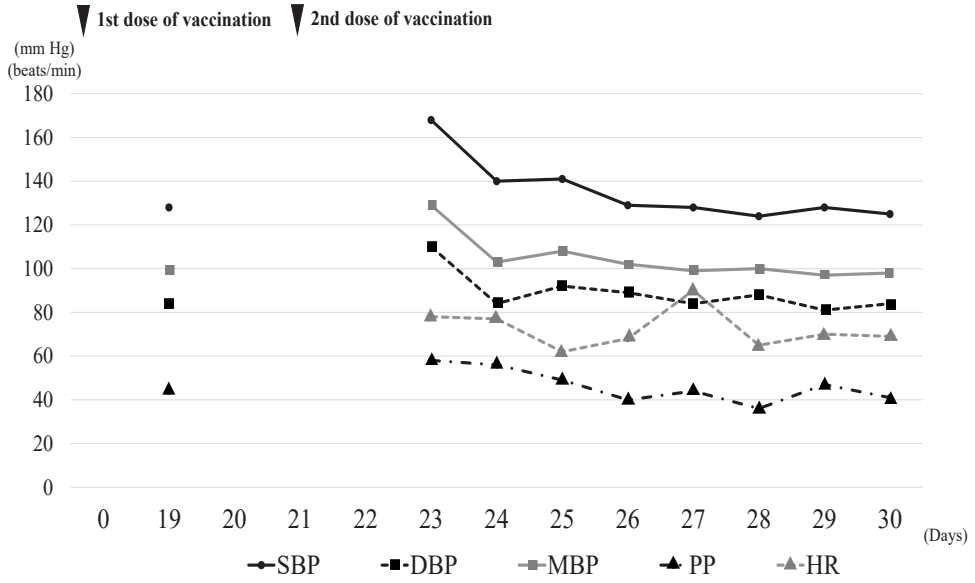


Fig. 4. Time course of change after vaccination (BNT162b2) — Blood pressure and heart rate in case 2. SBP, Systolic blood pressure; DBP, Diastolic blood pressure; PP, Pulse pressure; MBP, Mean blood pressure; HR, Heart rate.

positive for IgM antibodies.[11] After the second dose, all subjects tested positive for IgG antibodies (median: 15 days; IQR: 15–15 days), and again, none of them tested positive for IgM.[11] The positive cutoff value was defined as > 0.10 AU/mL for IgG and > 0.20 AU/mL for IgM in their report. It is possible that the increase in IgM antibody titer after BNT162b2 vaccination in Japanese people is low.

A longitudinal study, to quantify anti-S SARS-CoV-2 IgG and IgM (IgG-S and IgM-S) in health care worker recipients of the BNT162b2 vaccine, reported that there were three patterns of responses: 1) IgG positive/IgM negative (36.1%), 2) coordinated IgM-S/IgG-S responses appearing at the second dose (37.4%), and 3) IgM appearing after IgG (26.3%).[12] According to their study, IgG-S positive/IgM-S negative responses could be recruitment of cross-coronavirus immunity by vaccination.[12]

Moreover, cross-reactive immune responses to SARS-CoV-2 were observed in subjects with previous infection by common cold coronaviruses.[13,14] Furthermore, it has been reported that most Japanese people are also genetically predisposed to recognize immunogenic protein fragments shared between SARS-CoV-2 and the common cold coronaviruses.[15] In the absence of vaccination or previous infection with the target infectious disease, the immune response to a viral or bacterial attack is rapidly activated, resulting in a high susceptibility to fever and other immune reactions. The patient in Case 2 in this study showed a typical immune response with the IgM level elevation followed by the IgG level elevation. On the other hand, the IgG positive/IgM negative pattern in the patient in Case 1 suggests the possibility of an antibody response associated with the low elevation of IgM titers in Japanese individuals or cross-reactivity against common cold coronaviruses. Additionally, the negative IgM antibody result in Case 1 may have been due to the limited sensitivity of the antibody test kit. Further studies are required to determine the mechanisms underlying the negative result.

Differences in adverse reactions between recipients are also important to consider. A higher rate of adverse reactions has been reported after the second dose than after the first dose.[1,4] In this study, both participants experienced more side effects after the second dose. However, the inflammatory response, which is an adverse reaction to vaccination, is a necessary process, and the inflammation induced by vaccines stimulates the immune system, resulting in the acquisition of immunity.[16] The CRP levels in both cases increased after the first and second doses, indicating a positive immune system response. In this study, the CRP level of the patient in Case 2 increased to 2.01 mg/dL, followed by stage III hypertension, suggesting that an elevated inflammatory response, measured by CRP, may manifest as an adverse reaction.

A study that compared the severity of adverse reactions to the BNT162b2 vaccine with the levels of TNF-α, a proinflammatory cytokine, suggested that the higher the TNF-α levels, the more severe the systemic reactions.[9] In our study, blood pressure elevation and systemic reactions were severe in Case 2, suggesting that the TNF-α levels of the patient in Case 2 might have increased after vaccination, resulting in severe systemic reactions. Thus, the elevated CRP levels of the patient in Case 2 could be associated with the elevated TNF-α levels in response to vaccination.

Psychological factors have also been reported to be associated with the adverse reactions to COVID-19 vaccination. A systematic review and meta-analysis reported that significantly more adverse reactions were observed in the vaccine group compared with the placebo group, but nocebo responses accounted for 76.0% of systemic reactions after the first dose, and for 51.8% after the second dose.[17] It is important to keep in mind that the adverse reactions to COVID-19 vaccinations can be caused by psychological factors such as anxiety and concern about the vaccine.

A study by Kageyama et al. examined positive and negative correlations between an elevation of antibody titers after BNT162b2 vaccination and underlying conditions/medications.[18] In their study, factors associated with antibody titers after vaccination were analyzed and no correlation was found between antibody titers and thyroid disease, diabetes mellitus, asthma, and atopic dermatitis, whereas a correlation was found with medication use such as anti-allergic drugs, immunosuppressants, and corticosteroids.[18] In line with our observation in Case 1, their study reported that antibody titers did not correlate with hypertension or with antihypertensive drugs.[18] On the other hand, as we observed in Case 2 with the participant who was using anti-allergic drugs, their study also reported a positive correlation between antibody titers and medication for allergy.[18] The presence or absence of underlying medical conditions does not appear to be associated with antibody titers, but caution should be exercised when using some medications. Among subjects with comorbidities such as hypertension, BNT162b2 is highly effective in preventing the onset of disease.[1] However, exacerbation of underlying medical conditions after COVID-19 vaccination was observed in

patients with thyroid diseases, diabetes mellitus, lung diseases, and skin diseases.[19-22] It can be speculated that the presence of underlying medical conditions may increase side effects, but since most of the studies are case reports and case series, future large-scale studies are needed to confirm this matter.

It is a noteworthy observation that our patient in Case 2 developed stage III hypertension after the second dose, which was almost normalized without therapeutic intervention. A case series, similarly, found that nine patients developed grade III hypertension within minutes of receiving the BNT162b2 vaccine.[23] Possible explanations included pain, the "white coat effect," a reaction to vaccine components, and an interaction between the S-protein in the vaccine and angiotensin-converting enzyme 2 (ACE2) in the recipients.[23] Similarly in the patient in Case 2, we observed abnormally elevated mean and diastolic blood pressure coupled with a relatively low heart rate, suggesting increased peripheral vascular resistance. This is attributable to sympathetic nerve activation, renin-angiotensin system activation, atherosclerosis, and other factors.[24] The relationship between COVID-19 and the renin-angiotensin system has been the main focus of attention, as SARS-CoV-2 enters cells via the ACE2 receptor.[25] It has been suggested that when SARS-CoV-2 enters cells, the virus is endocytosed together with ACE2 receptors, downregulating ACE2 expression, promoting the accumulation of angiotensin II (Ang II), and inducing the production of inflammatory cytokines.[25] It is possible that after vaccination, the binding of vaccine-generated spike proteins to ACE2 receptors increased Ang II levels, inducing peripheral vasoconstriction resulting in elevated blood pressure. However, we were unable to determine the mechanism from the available data.

Subsequent research shows that hypertension was reported as an adverse drug reaction to COVID-19 vaccines.[26] Although it is not possible to establish a causal relationship between vaccination and hypertension based on the available data, we recommend that COVID-19 vaccine recipients have their blood pressure monitored for two days after vaccination.

Conflicts of Interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Research Ethics

This study was approved by the Ethical Review Board of the Faculty of Medicine, Fukui University (approval number: 20200023) and was carried out in accordance with the principles of the Declaration of Helsinki.

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