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Short Communications

**Anti-spike protein antibody responses to BNT162b2 mRNA vaccine: a single-center survey in a COVID-19 non-epidemic area in Japan**

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**Abbreviations**

ACE2: angiotensin-converting enzyme 2; COVID-19: coronavirus disease 2019; mRNA: messenger ribonucleic acid; RBD: receptor-binding domain; SARS-CoV-2: severe acute respiratory coronavirus 2; SD: standard deviation.
Abstract

Background
There are a few reports on antibody responses after a two-dose BNT162b2 vaccination in non-epidemic areas. We evaluated this phenomenon.

Methods
A total of 344 healthcare workers were vaccinated, and the serum anti-receptor-binding domain (RBD) antibody concentrations before and after two weeks following the two-dose BNT162b2 vaccination were measured using electro chemiluminescence immunoassay system.

Results
Before vaccination, the antibody titers of all participants were less than 0.6 U/mL. After two doses of the BNT162b2 vaccine injection in 342 participants (2 excluded), the average (±standard deviation) serum anti-RBD antibody titers were 2324±1739 U/mL. Antibody levels in females and males were 2443±1833 U/mL and 1908±1287 U/mL, respectively (p=0.037). Additionally, a 99.7% seroconversion rate was observed.

Conclusion
In a non-epidemic area, two BNT162b2 doses induced a satisfactory antibody response, and the antibody concentrations in females were higher than in males.

Keywords
COVID-19; SARS-CoV-2; mRNA vaccine; receptor-binding domain; spike protein.
Highlights

• Anti-spike protein antibody concentration was elevated by BNT162b2 vaccination.
• In a non-epidemic area, two BNT162b2 vaccine doses elicited good antibody responses.
• The seroconversion rate was 99.7%.
• Antibody responses to BNT162b2 in Asian females were higher than Asian males.

Introduction

The novel severe acute respiratory coronavirus 2 (SARS-CoV-2), was first identified in Wuhan, China and is the causative agent of the coronavirus disease 2019 (COVID-19) pandemic which began in December 2019 [1]. The receptor-binding domain (RBD) in the spike protein of SARS-CoV-2 can bind to the angiotensin-converting enzyme 2 (ACE2), the functional host receptor on host cell surfaces, to enter host cells [2].

Messenger ribonucleic acid (mRNA) vaccines against COVID-19 including the BNT162b2 (Pfizer/BioNTech) and mRNA-1273 (Moderna) were developed and are currently being administered worldwide. BNT162b2 contains a lipid nanoparticle-formulated, nucleoside-modified mRNA which encodes the full-length spike protein of SARS-CoV-2 [3]. The Phase 2/3 clinical trial revealed that BNT162b2 was 95% effective in preventing COVID-19 [4].

In Japan, BNT162b2 vaccination began in February 2021, but there have been a few reports on the vaccine’s efficacy in the Japanese population. In this study, we investigated the anti-spike protein antibody responses to BNT162b2 mRNA vaccination at our hospital in Hiroshima Prefecture in Japan, a non-epidemic area.
**Material and methods**

**Study design**

This was a single-center, prospective, observational investigation of the antibody responses to SARS-CoV-2 mRNA vaccination. This study was approved by the clinical ethics committees of Medical Corporation JR Hiroshima Hospital (approval number: 2020-56; approval data: March 9, 2021). We investigated 344 healthcare workers vaccinated with BNT162b2 and measured their serum anti-RBD antibody titers. The subjects’ provided informed consent before blood collection and vaccination. Two doses of BNT162b2 (Pfizer/BioNTech) intramuscular injection were administered three weeks apart. Adverse events were self-reported on the pre-vaccine interview of the third dose of BNT162b2, which was administrated 8 months after the second dose. Blood was collected before vaccination and two weeks after the final BNT162b2 injection. Subject demographics (age, sex and results of SARS-CoV-2 antigen tests) were obtained from the hospital’s medical records. The staffs of our hospital receive the salivary quantitative SARS-CoV-2 antigen test using the Lumipulse SARS-CoV-2 Ag system (Fujirebio, Tokyo, Japan) at random.

**Measurement of anti-spike protein antibody with electro chemiluminescence immunoassay (ECLIA)**

Serum antibody titers were measured using Elecsys Anti-SARS-CoV-2 spike immunoassay on a Cobas 6000 e601 module (Roche Diagnostics, Rotkreuz, Switzerland). Antibodies in this system can react to the RBD in SARS-CoV-2 spike protein. They were correlated with results from the cPass SARS-CoV-2 neutralization antibody detection kit (Genscript, Netherlands), which was available for a surrogate neutralization test based on
the ACE2-spike protein interaction [5,6]. Values ≥15 U/mL were considered positive for ≥20% neutralization with a positive predictive value of 99.10% [6].

**Statistical analysis**

The Mann-Whitney U test determined the differences between two groups. Single regression analyses using the Pearson’s correlation coefficient were also performed. Data processing and analyses were conducted using the Graph Pad Prism 9 software program (GraphPad Software, Inc., San Diego, CA, USA). P<0.05 was considered statistically significant. Results are presented as average ± standard deviation (SD).

**Results**

**Antibody response against spike protein after BNT162b2 vaccination**

None of the 344 healthcare workers was previously diagnosed with COVID-19 through SARS-CoV-2 antigen quantitative testing. All participants were Asian, and the mean age was 41.5±11.7 years. There were 268 (77.9%) female participants. Before the first BNT162b2 vaccination, the anti-RBD antibody titers for all participants were either undetectable or 0.6 U/mL (Table 1). Notably, two female participants did not able to receive the second vaccine dose due to vaccine-associated side reactions. After two vaccination doses, seroconversion was observed in 99.7% (341 out of 342), and the mean serum anti-RBD antibody titers was 2324±1739 U/ml (Table 1; Figure 1). After the second vaccination, seven participants were infected with COVID-19. All of their symptoms were mild. None of the participants went to the hospital for hyperglycemia.

**Females have higher antibody responses to BNT162b2**
The mean serum anti-RBD antibody titers between vaccinated females and males were 2443 \pm 1833 \text{ U/mL} and 1908 \pm 1287 \text{ U/mL}, respectively, and there was a significantly difference between these two groups (p=0.037; Figure 2). These results indicated that antibody titers were higher in Japanese adult females than in males. We also measured the correlation coefficient between serum anti-RBD antibody levels and the healthcare workers’ age. There was no correlation between these two factors (p=0.30; Figure 3).

**Discussion**

Trained immunity by live attenuated vaccines (e.g., BCG), innate immunity, and T cell responses have been reported to be essential host immunological systems against COVID-19 [7-9]. Though the host immunological mechanisms against SARS-CoV-2 are still unclear, humoral immunity may also play a significant role in fighting SARS-CoV-2 infections. Bamlanivimab (LY-CoV555), an anti-RBD monoclonal antibody, was ineffective in hospitalized patients with COVID-19 but reduced the incidence of SARS-CoV-2 infections in residents and staff of skilled nursing and assisted living facilities [10,11]. Therefore, we hypothesize that the measurement of anti-RBD antibody concentrations is useful in COVID-19 vaccination follow-ups. Anti-RBD antibodies have the potential to protect the host by neutralizing the S protein and exhibiting antibody-dependent cell-mediated cytotoxicity (ADCC) activity [12]. One hypothesis is that the anti-RBD antibodies produce infection-enhancing activities [13]. However, follow-ups of our subjects showed that there were only 7 mild cases of COVID-19; therefore, the theory is not well supported by our data.

There are several methods to evaluate the effectiveness of vaccination against SARS-CoV-2 in individuals, including *ex vivo* interferon-gamma enzyme-linked immunospot
using CD4+ or CD8+ T cells, surrogate neutralization test, and serum anti-RBD/anti-spike protein antibody levels [5,14]. Here, we used the serum anti-RBD antibody titer system purchased from Roche, which can be used on several general-purpose machine in clinical laboratories. In Japan, we can also use the Abbott SARS-CoV-2 IgG II Quant-test system to detect serum anti-RBD antibodies, which has a good correlation with the above-mentioned system [15].

Reiterer M. et al. reported that patients with acute respiratory distress syndrome and COVID-19 had insulin resistance and hyperglycemia, independent of glucocorticoid treatment [16]. In our study, there were no incidences of hyperglycemia in participants who received the second dose. Therefore, we cannot determine whether there were any relationships with hyperglycemia in our study population.

Individuals previously infected with SARS-CoV-2 can boost their antibody response by a single BNT162b2 dose [17-19]. Most of the vaccination clinical trials and observational studies thus far have been performed in COVID-19 epidemic areas. Therefore, BNT162b2 vaccination efficiency may be affected by some factors in these areas. Our hospital is located in a non-epidemic area; thus, differences with epidemic area are expected. Notably, anti-RBD antibody concentrations prior to vaccination were extremely low. In this present study, we certified that two dose of BNT162b2 elicited adequate antibody response in a non-epidemic area as the rate of seroconversion was 99.7%.

Additionally, our data indicated that females had higher antibody levels after two vaccination doses when compared to males. Several factors may potentially influence their antibody titers after two doses of the SARS-CoV-2 vaccine. Non-Caucasian ethnicities have been associated with higher anti-spike antibody concentrations than Caucasian ethics groups [6]. Current smokers and older individuals were also independently
associated with lower antibody levels than non-smokers and younger individuals, respectively [6]. However, we did not detect a relationship between the antibody concentration and age in our study in Japan.

Conclusions

This is the first study to measure anti-spike antibody responses to BNT162b2 vaccination in a cohort from a non-epidemic area of Japan. Two BNT162b2 doses prompted sufficient antibody responses, and these responses were higher in Japanese females than in males.

Our study had several limitations. First, we conducted a single-center study, but a multi-center study would be needed for have provided more reliable data and information on risk factors. Secondly, we used ECLIA to measure antibody levels instead of a neutralizing assay, which is more biologically relevant. Lastly, we failed to collect more subject demographics such as smoking status, underlying diseases, and medications taken.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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Figure legends

**Figure 1 (Colored figure)**. Antibody response against the spike protein following two doses of the BNT162b2 vaccine.

Data are displayed as average (dotted line) and standard deviation.

**Figure 2 (Colored figure)**. Comparison of antibody response for the BNT162b2 vaccination in female and male healthcare workers.

Data are displayed as average (dotted line) and standard deviation.

* Mann-Whitney U test, p<0.05

**Figure 3**. The correlation between age and anti-spike protein antibody levels after the BNT162b2 vaccination.

Gray dotted line is a regression line.

Data was analyzed using Pearson’s correlation test.
Table1. Baseline characteristics for healthcare workers vaccinated with BNT162b2

|                           | Before vaccination | After the two-dose vaccination |
|---------------------------|--------------------|--------------------------------|
| Case number               | 344                | 342                            |
| Female (n, %)             | 268, 77.9%         | 266, 77.8%                     |
| Age (years)               |                    |                                |
| Total                     | 41.5±11.7          | 41.6±11.7                      |
| Female                    | 40.2±11.3          | 40.3±11.4                      |
| Male                      | 45.9±12.0          | 46.1±11.9                      |
| Antibody titer (U/mL)     | 0.4±0.01 *         | 2324.1±1739.0                  |

Data was displayed as average ± standard deviation.

* Undetectable cases were defined as 0.4 U/mL.
Anti-SARS-CoV-2 S antibody (U/mL)

Before
n = 344

After
n = 342

2324 ± 1739
Anti-SARS-CoV-2 S antibody (U/mL)

Female
n=266
2443 ± 1833

Male
n=76
1908 ± 1287

R²=0.003
p=0.31

* p=0.037
Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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