Poster # 1467

No correlation of neutralizing antibody titers against the Omicron variant after a booster dose of COVID-19 vaccines with subsequent breakthrough Omicron infections among healthcare workers

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Revised Abstract

Objectives: Data on the immune correlation of protection from breakthrough Omicron (B.1.1.529) infection in individuals who received booster dose of COVID-19 vaccines are lacking.
Methods: We enrolled healthcare workers (HCWs) without history of SARS-CoV-2 infection who received a booster mRNA vaccination at a tertiary hospital between November 2021 and December 2022 (Delta-dominant era). Breakthrough Omicron infection was identified by SARS-CoV-2 polymerase-chain-reaction (PCR), between February 1, 2022 and April 25, 2022 (Omicron-dominant era). Plasma levels of live-virus neutralizing antibodies were measured with the SARS-CoV-2 Omicron variant.
Results: A total 119 HCWs, including 56 (47%) in breakthrough group and 63 (53%) in non-breakthrough group, were enrolled. No significant difference in median (IQR) ID_{50} against Omicron at 2 weeks after booster was observed between the two groups (1781.9 [1499.5.0–4500.0] vs 2613.9 [1770.7–4498.6]; p = 0.10). There were no significant differences in median (IQR) ID_{50} against Omicron at 3 months after booster between the breakthrough group (442.2 [191.3–807.4]) and non-breakthrough group (462.4 [281.1–592.5]; p = 0.39), and in the decrease of neutralizing antibody titers between the two groups (β = −380.5 [SE, 680.6]; p = 0.58).
Conclusions: Neutralizing antibody titers against Omicron after the booster dose of COVID-19 vaccines was not correlated with subsequent breakthrough Omicron infections.

Introduction

Since November 2021, the Omicron variant (B.1.1.529) has been categorized as a variant of concern by the World Health Organization and has rapidly spread globally.[1] With the immune evasion potential of Omicron and vaccine-induced waning immunity, many countries administer a booster vaccine for individuals who have received a complete primary series of the coronavirus disease 2019 (COVID-19) vaccine.[2-4] Although a booster vaccination has demonstrated some protective effects against the Omicron variant infection, breakthrough infections frequently occur in booster-vaccinated individuals.[5,6] However, data on the immune correlation of protection against breakthrough Omicron infection in individuals who received booster COVID-19 vaccines are limited. Thus, this prospective cohort study aimed to compare humoral immune responses including neutralizing antibody titers against the Omicron variant and S1-specific antibody at 2 weeks and 3 months after a booster dose of COVID-19 mRNA vaccines, respectively, among healthcare workers (HCWs) who experienced Omicron breakthrough infections and those without Omicron infections.

Methods

1. Study participants and design

We enrolled HCWs without a history of SARS-CoV-2 infection, who received a COVID-19 booster vaccine after primary series at Asan Medical Center, a 2,700-bed tertiary hospital in Seoul, South Korea from November 2021 to December 2022 (Delta-dominant era). HCWs were provided two doses of ChAdOx1 nCoV-19 (ChAdOx1; AstraZeneca), BNT162b2 (Pfizer-BioNTech), or mRNA-1273 (Moderna) as primary series, followed by a booster dose of BNT162b2 or mRNA-1273.

We measured the serum level of neutralizing antibodies and S1-specific IgG antibodies at 2 weeks and 3 months, respectively, after booster vaccination. We compared humoral immune responses between HCWs with and without breakthrough SARS-CoV-2 infection at both time points. The study was approved by the institutional review board at Asan Medical Center (IRB No 2020-0298) and informed consent was obtained from all the participants.

2. Confirmation of SARS-CoV-2 infection

During the study period, all HCWs who had COVID-19-associated symptoms or epidemiologic links to confirmed COVID-19 patients were recommended to undergo SARS-CoV-2 PCR testing of their nasopharyngeal specimens to identify SARS-CoV-2 infections between 1 February and 25 April 2022 (Omicron-dominant era). A breakthrough Omicron infection was defined as the detection of SARS-CoV-2 infection by PCR testing through respiratory specimen during the Omicron-dominant era. We also performed serologic testing for SARS-CoV-2 infection through anti-SARS-CoV-2 nucleocapsid (N) protein antibody at 3 months after the booster vaccination to rule out asymptomatic COVID-19 among HCWs who never had confirmed SARS-CoV-2 infection.

3. Measurement of immune responses

A microneutralization assay with SARS-CoV-2 Omicron variant (B.1.1.529) was used to measure plasma levels of live-virus neutralizing antibodies and was performed in a Bio Safety Level (BSL)-3 laboratory at the Institut Pasteur Korea (Seongnam, South Korea). Briefly, a 100-tissue culture infective dose 50 (100 $TCID_{50}$) of SARS-CoV-2 Omicron variant (hCoV-19/Korea/KDCA447321/2021 NCCP 43408) provided by the Korea Disease Control and Prevention Agency was mixed with an equal volume of diluted plasma specimen, incubated at 37°C for 30 min, and added to Vero cells. After 96 h, the cytopathic effect of SARS-CoV-2 on the infected cells was measured and neutralizing antibody titer calculated as the reciprocal of the highest dilution of test plasma providing 50% neutralization (ID_{50}).

SARS-CoV-2 S1-specific IgG antibody titers were measured using an enzyme-linked immunosorbent assay (ELISA) developed in-house, details of which are described in a previous report.[7] Briefly, 2 mg/mL SARS-CoV-2 S1-His protein (SinoBiological, Beijing, China) was coated onto 96-well plates (MaxiSorp; Thermo Fisher Scientific, Waltham, MA) overnight at 4°C, and then the plates were blocked with 1% bovine serum albumin in phosphate buffered saline (PBS). Plasma diluted at 1:100 was added and incubated for 2 hours at room temperature. Horseradish peroxidase-conjugated anti-human IgG (Jackson Immunoresearch, West Grove, PA) were used as secondary antibodies. The data are presented as International Units per milliliter (IU/ml), which is standardized with reference pooled sera from International Vaccine Institute (Seoul, South Korea).To determine cut-off values for the ELISA, the mean and standard deviation (SD) of negative control plasma were measured, and cut-off values were defined as mean IU plus three-fold the SD value; the cut-off value was 10 IU/ml for IgG, as reported previously.[8,9]

SARS-CoV-2 N-specific IgG antibody titers were also assessed by ELISA. One mg/mL SARS-CoV-2 N-His protein (SinoBiological, Beijing, China) was coated onto 96-well plates (Thermo Fisher Scientific) overnight at 4°C, and then following procedures are same with S1-specific IgG ELISA. The data are presented as Absorbance Unit per milliliter (AU/mL). The results were considered as negative if the results were under 1.4 AU/ml, positive if the results were over 2.0 AU/ml, and borderline if the results were between 1.4 and 2.0 AU/ml.

Results

Study population

Among a total 127 fully vaccinated HCWs who agreed to blood sampling after booster vaccination, eight who had a history of COVID-19 were excluded (Figure 1). Of them, 56 (47%) experienced breakthrough Omicron infection at a median of 124 days (IQR 99.5–150) after booster vaccination (breakthrough group), and the remaining 63 (53%) did not experience Omicron infection (non-breakthrough group). Of these 63 HCWs who had never been confirmed positive for SARS-CoV-2 by PCR testing, four (3 ChAdOx1-BNT162b2 and 1 mRNA-1273) HCWs who had positive anti-SARS-CoV-2 N protein antibody at 3 months after booster vaccination were excluded from the non-breakthrough infection group (Figure 1).

Figure 1. Study flowchart

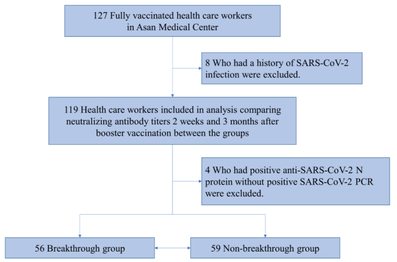


Table 1. Characteristics between the breakthrough group and non-breakthrough group

	Breakthrough group (n = 56)	Non- breakthrough group (n = 59)	p value
Age, median (range), years	35 (22–59)	33 (24–64)	0.22
Sex			
Female	41 (73)	45 (76)	0.87
Male	15 (27)	14 (24)	
Type of vaccination,			
AZ-AZ-PF	34 (61)	23 (39)	0.01
PF-PF-PF	21 (37)	27 (46)	
MO-MO-MO	1 (2)	9 (15)	
Interval from second dose to booster dose, median (IQR), days	182 (175–196)	182 (169.5–197)	0.82
Interval from booster dose to infection, median (IQR), days	124 (99.5–150)	Not applicable	
COVID-19 severity			
Asymptomatic	19 (34)	Not applicable	
Mild	37 (66)	Not applicable	

Data are presented as no. (%) of individuals unless otherwise indicated. Abbreviations: IQR, interquartile range; AZ, ChAdOx1 nCoV-19; PF, BNT162b2; MO, mRNA-1273.

Figure 2. Comparison of neutralizing antibody and S1-specific IgG antibody titers between the breakthrough group and non-breakthrough group

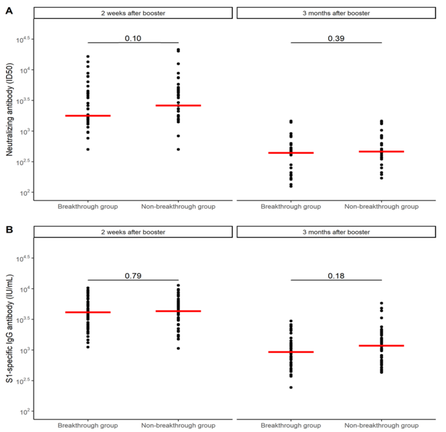
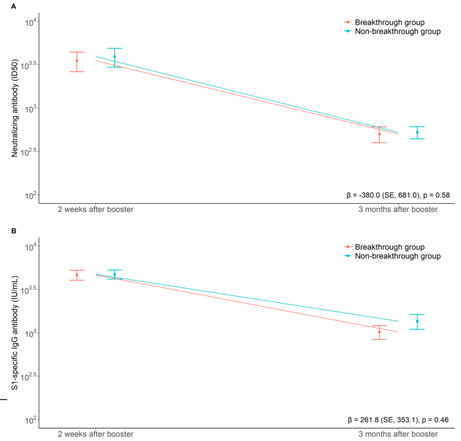


Figure 3. Time interaction of neutralizing antibody and S1-specific IgG antibody titers according to Omicron (B.1.1.529) breakthrough infections.



Discussion

In this study involving HCWs who received booster COVID-19 mRNA vaccines after the primary series, we compared the humoral immune response between HCWs who experienced Omicron breakthrough infections and HCWs without Omicron infections. No significant differences in neutralizing and S1-specific IgG antibody titers at 2 weeks and 3 months after booster vaccination were observed between the breakthrough group and non-breakthrough group. In addition, no significant difference in the waning slope of neutralizing and S1-specific IgG antibody titers in the time interaction was observed between the groups. Therefore, no immune correlation of protection against breakthrough Omicron infection was identified in individuals who received booster COVID-19 vaccines.

As Delta (B.1.617.2) became the dominant variant in some countries, breakthrough infections after mRNA and adenovirus-vectored vaccinations have been widely reported.[5,10-13] However, limited studies accessed the immune correlation of protection for neutralizing antibody titer against the circulating SARS-CoV-2 strains. In a prospective cohort study from Israel, the risk of breakthrough infections with SARS-CoV-2 was correlated with levels of neutralizing antibodies against ancestral SARS-CoV-2, and the peak titers of neutralizing antibody were more likely associated with the risk of breakthrough infection than the peri-infection titers of neutralizing antibody.[11] However, few data regarding immune correlation of protection against breakthrough Omicron infection after booster vaccination are available. Here, no significant difference in humoral immunity was observed between the breakthrough group and non-breakthrough group. Therefore, we were unable to determine the cut-off value of neutralizing antibody titers for preventing Omicron infection. This finding is not consistent with previous studies supporting the assumption that the presence of the neutralizing antibodies would correlate with the protection from SARS-CoV-2 infection.[14,15]

The discrepancy may be due to some possibilities. First, due to the relatively small sample size, we could not find any statistically significant differences. However, this possibility might not be high or at least the difference of neutralizing antibody titers between two groups may be marginal, considering that not only there was no difference in neutralizing antibody titers measured during outbreak of Omicron, where most infections occurred, but also there was no difference in the decreasing slope of neutralizing antibody titers over time between the groups. Second, unlike the ancestral strain or the Delta variant (B.1.617.2), Omicron infection is more likely to be confined to the upper respiratory tract,[16,17] and maintaining a steep concentration gradient with much higher plasma neutralizing antibody titer is required to prevent the cases of such mild infection.[15] Since all the breakthrough Omicron infection cases in our study was asymptomatic or mild illness, the result could be explained by the possibility that the level of neutralizing antibody induced after booster dose is not sufficiently high to prevent mild disease. However, since the preventive effect on severe COVID-19 caused by lower respiratory tract infection could be obtained by a relatively lower neutralizing antibody titer,[15] further studies regarding immune correlation of protection against severe Omicron infection are needed. In addition, since T-cell immune response and humoral immunity may likely play an important role in preventing severe COVID-19,[18,19] additional studies exploring cellular immune response against breakthrough Omicron infection or progression to severe diseases are needed.

It is worth noting that the HCWs in the breakthrough group more likely received the two-dose ChAdOx1 nCoV-19 followed by BNT162b2 and the HCWs in non-breakthrough group more likely received the three-dose mRNA-1273. This suggests that the preventive effect against breakthrough Omicron infection may differ depending on the type of vaccines. This result is consistent with that in reported previous studies, revealing higher vaccine efficacy of the mRNA-1273 vaccine than the BNT162b2 vaccine before the emergence of the Omicron variant.[20] Further studies are needed to establish different vaccine effectiveness of booster dose against breakthrough Omicron infection according to the type of vaccines.

This study had some limitations. First, although we measured the neutralizing antibody titers using a microneutralization assay with SARS-CoV-2 Omicron variant (B.1.1.529), which was initially the prevalent sublineage of the Omicron variant, sublineage BA.2 has surpassed sublineage BA.1 in South Korea after April 2022. Therefore, measuring neutralizing antibody titers against SARS-CoV-2 Omicron variant (B.1.1.529) could be limited in evaluating immune responses among HCWs infected with Omicron variant (BA.2). Second, since the breakthrough group and non-breakthrough group were not randomized, the level of exposure to the SARS-CoV-2 Omicron variant between the groups may not have been the same. Thus, the HCWs living more carefully may not experience breakthrough Omicron infection even if the neutralizing antibody titers were relatively low, and these behavioral factors were not measured in this study. Last, HCWs have a higher level of exposure to SARS-CoV-2 than the general population as HCWs attend to patients who are undiagnosed as having COVID-19 until proper isolation. This different level of exposure to SARS-CoV-2 Omicron variant could introduce some bias toward the null and some caution is needed for generalizing our findings into general population.

In conclusion, neutralizing antibody titers against Omicron at 2 weeks and 3 months after the booster dose of COVID-19 vaccines was not correlated with subsequent breakthrough Omicron infections.

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