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IMMUNE NETWORK

Original Article

Low Neutralizing Activities to the Omicron Subvariants BN.1 and XBB.1.5 of Sera From the Individuals Vaccinated With a BA.4/5-Containing Bivalent mRNA Vaccine

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ABSTRACT

The continuous emergence of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) variants has provided insights for updating current coronavirus disease 2019 (COVID-19) vaccines. We examined the neutralizing activity of Abs induced by a BA.4/5-containing bivalent mRNA vaccine against Omicron subvariants BN.1 and XBB.1.5. We recruited 40 individuals who had received a monovalent COVID-19 booster dose after a primary series of COVID-19 vaccinations and will be vaccinated with a BA.4/5-containing bivalent vaccine. Sera were collected before vaccination, one month after, and three months after a bivalent booster. Neutralizing Ab (nAb) titers were measured against ancestral SARS-CoV-2 and Omicron subvariants BA.5, BN.1, and XBB.1.5. BA.4/5-containing bivalent vaccination significantly boosted nAb levels against both ancestral SARS-CoV-2 and Omicron subvariants. Participants with a history of SARS-CoV-2 infection had higher nAb titers against all examined strains than the infection-naïve group. NAb titers against BN.1 and XBB.1.5 were lower than those against the ancestral SARS-CoV-2 and BA.5 strains. These results suggest that COVID-19 vaccinations specifically targeting emerging Omicron subvariants, such as XBB.1.5, may be required to ensure better protection against SARS-CoV-2 infection, especially in high-risk groups.

Keywords: Antibodies, neutralizing; COVID-19; SARS-CoV-2; Vaccine

INTRODUCTION

Since its emergence in late 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) devastatingly impacted public health, economy, and society. The Omicron variant (B.1.1.529), initially designated as a variant of concern in November 2021, has replaced previous variants; moreover, most of the currently circulating SARS-CoV-2 strains are

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Conflict of Interest
The authors declare no potential conflicts of interest.

Abbreviations
CI, confidence interval; COVID-19, coronavirus disease 2019; DW, distilled water; GMFR, geometric mean fold rise; GMT, geometric mean titer; IRB, Institutional Review Board; ML, maximum likelihood; N, nucleocapsid; nAb, neutralizing Ab; NCCP, National Culture Collection for Pathogens; ND50, 50% neutralizing dose; RT, room temperature; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; VRNT, virus reduction neutralization test.

Author Contributions
Conceptualization: Noh JY; Formal analysis: Nham E, Kim J, Hwang SY; Investigation: Nham E, Kim J, Lee J, Park H, Kim J, Lee S, Choi J, Kim KT, Park MS, Noh JY; Methodology: Nham E, Kim J, Song JY, Cheong HJ, Kim WJ, Park MS, Noh JY; Software: Nham E, Kim J, Park MS, Noh JY; Validation: Nham E, Kim J, Park MS, Noh JY; Writing - original draft: Nham E, Kim J, Park MS, Noh JY; Writing - review & editing: Nham E, Kim J, Lee J, Park H, Kim J, Lee S, Choi J, Kim KT, Yoon JG, Hwang SY, Song JY, Cheong HJ, Kim WJ, Park MS, Noh JY.

descendants of it (1). Owing to this immune-evasive variant, vaccines which were developed based on the ancestral SARS-CoV-2 are significantly less effective in preventing SARS-CoV-2 infection (2). To combat this, bivalent coronavirus disease 2019 (COVID-19) vaccines containing both ancestral and Omicron subvariants, BA.1 or BA.4/5, were rapidly developed (3,4). In Korea, bivalent COVID-19 vaccines became available since October 2022. However, its coverage rate is substantially low, even in populations at a high risk of severe COVID-19. As of June 21, 2023, only 35% of people aged 60 years or older and 29% of individuals with immunocompromised conditions have received bivalent vaccines (5).

The genetic and antigenic evolution of SARS-CoV-2 is ongoing, and Omicron subvariants have continuously emerged. BN.1, the subvariant of BA.2.75, accounted for 60% of the total sequenced SARS-CoV-2 in mid-February 2023 after its first detection in late 2022 in Korea (6). As of mid-June 2023, the dominant lineage worldwide is XBB, a recombinant of BA.2.10.1 and BA.2.75. Among its sublineages, XBB.1.5, XBB.1.16, and XBB.1.9 are most prevalent (7,8). Considering predominance of XBB.1.5 and its high degree of immune evasion, the World Health Organization has recommended XBB.1.5 or XBB.1.16 as an Ag composition of updated COVID-19 vaccine for 2023–2024 (9). The United States Food and Drug Administration also advised vaccine manufacturers that vaccines should comprise the monovalent XBB.1.5 (10).

Here, we report the neutralizing activities of Abs induced by a BA.4/5-containing bivalent booster against two later Omicron subvariants, BN.1 and XBB.1.5, which have been prevalent in Korea following the circulation of BA.5.

MATERIALS AND METHODS

Study population and design

We recruited individuals aged 19 years and older who had completed a primary series and monovalent booster dose of the COVID-19 vaccination schedule at least 90 days prior and were planning to receive a BA.4/5-containing bivalent mRNA booster (4th dose). Individuals who had severe anaphylaxis after COVID-19 vaccination, those with serious allergies to one or more vaccine components, and those who received Ab treatment for COVID-19 within 90 days were excluded.

Due to the high percentage of people who had contracted COVID-19, we divided the study population into two groups: 1) individuals with a known history of SARS-CoV-2 infection or anti-SARS-CoV-2 nucleocapsid (N) Ab positivity (previously infected group) and 2) individuals without a history of SARS-CoV-2 infection and negative for anti-SARS-CoV-2 N Ab (infection-naïve group). Whole blood samples were collected 1) before receiving the bivalent vaccine, 2) one month later, and 3) three months later. Anti-N IgG and neutralizing Abs (nAbs) against the ancestral SARS-CoV-2, BA.5, BN.1, and XBB.1.5 were measured at each time point (**Fig. 1A**).

Phylogenetic analysis

For the phylogenetic analysis, 257 complete and high-coverage SARS-CoV-2 sequences were downloaded from the GISAID EpiCoV database. Sequence alignment was conducted using MAFFT. The maximum likelihood (ML) tree was constructed using MEGA X with 1,000 bootstrap replicates based on the general time reversible model with gamma distribution and invariant sites (G+I) nucleotide substitution model. For the ML tree, an initial tree was

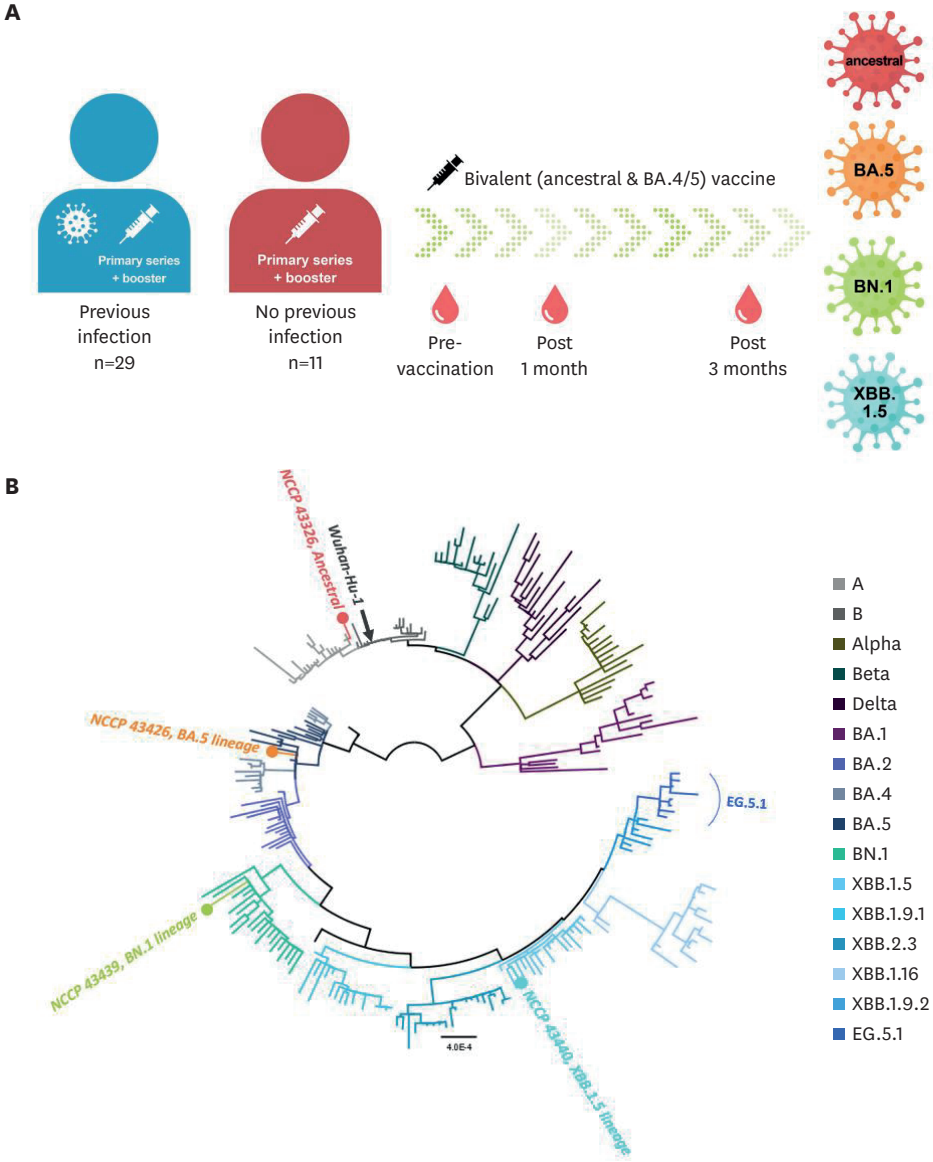

Figure 1. Study design and phylogenetic tree of SARS-CoV-2 including variants used in this study. (A) Study design. (B) ML tree of SARS-CoV-2. ML tree indicates each lineage of SARS-CoV-2. Sequences from each virus for VRNT are labeled with red (ancestral; Wuhan-like A lineage), orange (BA.5 lineage), light green (BN.1 lineage), and cyan (XBB lineage) branches and additionally pointed with circles. The scale bar indicates nucleotide substitutions per site.

obtained by the neighbor-joining and BioNJ methods. FigTree (v1.4.4) was used for ML tree visualization.

Measurement of anti-SARS-CoV-2 N Ab

To identify serological evidence of SARS-CoV-2 infection, the serum level of anti-SARS-CoV-2 N Ab was measured. Elecsys® Anti-SARS-CoV-2 (Roche Diagnostics, Basel, Switzerland), an electrochemiluminescence immunoassay, was performed using the cobas® 8000 analyzer (Roche Diagnostics). A cut-off index ≥1.0 was considered positive.

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Neutralization test

Cell lines

Vero cells (Vero01WCB-1201, Ministry of Food and Drug Safety, Cheongju, Korea) were maintained in DMEM (Gibco #41966029, Carlsbad, CA, USA) supplemented with 10% FBS (SERANA #S-FBS-US-015, Europe, Pessin, Germany) and 1% penicillin/streptomycin (10,000 U/ml, Gibco # 15140-122). Cells were grown at 37°C and 5% CO_2-humidified incubator and passaged every 2–3 days.

Viruses

The following four SARS-CoV-2 viruses were kindly provided by the National Culture Collection for Pathogens (NCCP), Korea Disease Control and Prevention Agency: SARS-CoV-2/human/KOR/NCCP 43326/2020 (ancestral; Wuhan-like A lineage, GenBank accession#: MW466791), SARS-CoV-2/human/KOR/NCCP 43426/2022 (Omicron BA.5, GISAID accession#: EPI_ISL_13086516), SARS-CoV-2/human/KOR/NCCP 43439/2023 (Omicron BN.1), and SARS-CoV-2/human/KOR/NCCP 43440/2023 (Omicron XBB.1.5). The viruses were passaged into Vero cells. All experiments using SARS-CoV-2 isolates were performed in a Biosafety Level 3 laboratory at the Korea University College of Medicine (KCDC-18-3-02).

Reagents and Abs

The following reagents and Abs were purchased commercially and used in virus reduction neutralization test (VRNT): PBS (Himedia #TL1101, India), Tween-20 (Sigma-Aldrich #P7949, St. Louis, MO, USA), 4% paraformaldehyde (PFA, T&I #BPP-9004, Wonju, Korea), Triton-X 100 (Sigma-Aldrich #93443), BSA (Bioshop #ALB001. 100, Burlington, Canada), carboxymethylcellulose sodium (CMC, Sigma-Aldrich #C9481), anti-SARS-CoV-2 spike glycoprotein Ab (Abcam #ab272504, Cambridge, UK), goat anti-rabbit IgG (H+L) cross-absorbed secondary Ab, Alexa Flour™ 488 (Thermo Fisher Scientific #A-11008, Waltham, MA, USA), DAPI (Sigma-Aldrich #D9452).

VRNT

High-throughput VRNT assay was developed with reference to the existing VRNT assay for dengue and respiratory syncytial viruses to assess nAb titers against SARS-CoV-2 in 96-well cell-carrier ultra-plates (PerkinElmer, Waltham, MA, USA) (11,12). One day before the neutralization assay, 10,000 cells per well were added to 96-well plates and tapped lightly on each side to evenly distribute the cells in the well. The serum was heat inactivated at 56°C, 30 min before use. The serum was 2-fold serially diluted in 96-well plates (SPL Life Science #34196, Pocheon, Korea) using PBS. SARS-CoV-2 was diluted in PBS with the optimal MOI of each viral strain that was determined by the linear regression of SARS-CoV-2 object counts against SARS-CoV-2 input. An equal volume of diluted SARS-CoV-2 was added to the serially diluted serum and incubated at 37°C and 5% CO_2 for 1 h for virus neutralization. The virus-serum mixture was then transferred to the 96-well plates containing Vero monolayer and incubated for 1 h at 37°C. After 1 h incubation, virus-infected cells were overlaid with CMC medium (0.8%), and incubated for 20–48 h at 37°C. At 20–48 h post-infection, the cells were fixed with 4% PFA for 30 min. After removing the PFA solution, the cells were washed thrice with PBS-Tween 20 solution (PBS containing 0.1% Tween 20; PBS-T). Cells were then incubated with permeabilization solution (PBS containing 0.5% Triton X-100) for 15 min at room temperature (RT). The permeabilization solution was removed and the cells were washed three times with PBS-T. After permeabilization, the cells were incubated with blocking buffer (PBS containing 0.1% Tween 20 and 1% BSA) for 30 min at RT. The cells

were then incubated with rabbit anti-SARS-CoV-2 spike Ab (1:1,000 dilution in blocking buffer) for 1 h at RT, followed by incubation with goat anti-rabbit Alexa Fluor™ 488 (1:1,000 dilution in PBS containing 0.1% Tween 20 and 0.1% BSA) for 1 h at RT and DAPI for 5 min at RT. Washing with PBS-T for 5 min at RT was performed three times between each step. After washing DAPI with three times PBS-T, 50 μl of distilled water (DW) was added, and finally, cells were visualized with an Operetta high-content imaging system (PerkinElmer) at 10× magnification at the Vaccine Innovation Center of Korea University. The number of virus-infected and DAPI-positive cells was calculated using the Harmony software in the Operetta CLS system (PerkinElmer). The 50% neutralizing dose (ND_{50}) titer was calculated using the Karber formula: log10 ND_{50} = m-Δ(∑p-0.5) (13). Every 96-well plate included virus control, mock-infected cells as a negative control, and two positive controls.

Statistical analysis

The geometric mean titer (GMT) and geometric mean fold rise (GMFR) with a 95% confidence interval (CI) were calculated for comparison of the ND_{50} titers using the VRNT. The normality of the log-transformed nAb titer distribution was examined by the Shapiro-Wilk normality test. Comparison of nAb GMT against certain variants across time points and comparison of nAb titers against different variants at certain time point were performed by the paired *t*-test or Wilcoxon signed rank test. Comparison of nAb GMT between previously infected and infection-naïve groups was performed by the Wilcoxon rank sum test. Multiple comparison adjustment was performed by Bonferroni correction. All statistical analyses were performed using the SAS (version 9.4; Cary, NC, USA) and SPSS (version 26; IBM, Armonk, NY, USA).

Ethics statement

All the participants provided written informed consent. This study was approved by the Institutional Review Board (IRB) of Korea University Guro Hospital (IRB No:2022GR0423).

RESULTS

Among the 40 subjects, the median age was 36 years (interquartile range, 29–40 years) and 80% were women. None of the participants reported any underlying immunocompromised conditions. Among these, 29 were previously infected with SARS-CoV-2: 28 individuals with a known history of SARS-CoV-2 infection and anti-N IgG positivity and one individual without a history of SARS-CoV-2 infection and positive for anti-N IgG. In the SARS-CoV-2 infection-naïve group, two individuals were infected with SARS-CoV-2 and seroconverted to anti-N IgG positivity after BA.4/5-containing bivalent vaccination.

BA.4/5-containing bivalent mRNA vaccine elicited significant increase in nAb against the ancestral SARS-CoV-2 and Omicron subvariants one month after vaccination: GMFR of nAb for the ancestral SARS-CoV-2, 3.9 (95% CI, 3.1–5.0); for BA.5, 6.5 (95% CI, 4.9–8.6); for BN.1, 5.2 (95% CI, 4.1–6.5); for XBB.1.5, 4.0 (95% CI, 3.2–5.2). A similar trend was also observed three months after vaccination: GMFR for the ancestral SARS-CoV-2, 1.4 (95% CI, 1.1–1.8); for BA.5, 5.3 (95% CI, 3.9–7.2); for BN.1, 3.8 (95% CI, 3.0–4.9); for XBB.1.5, 3.0 (95% CI, 2.3–4.0) (**Table 1**).

The nAb titers against BN.1 and XBB.1.5 were lower than those against the ancestral SARS-CoV-2 and BA.5 at baseline. One month after bivalent vaccination, compared to nAb titer

Table 1. ND_{50} against the ancestral SARS-CoV-2, BA.5, BN.1, and XBB.1.5 before and after BA.4/5-containing bivalent mRNA vaccination

Virus	Time point	Overall (n=40)*		Previously infected (n=29)†		Infection-naïve (n=11)*	
		GMT (95% CI)	GMFR (95% CI)	GMT (95% CI)	GMFR (95% CI)	GMT (95% CI)	GMFR (95% CI)
Ancestral SARS-CoV-2	Pre-vaccination	394.9 (284.4–548.2)	Ref.	628.2 (467.8–843.5)	Ref.	116.1 (89.5–150.7)	Ref.
	1 month post-vaccination	1,603.0 (1,338.1–1,920.3)	3.9 (3.1–5.0)	1,804.3 (1,455.5–2,236.0)	2.9 (2.3–3.6)	1,137.4 (868.0–1,490.3)	9.8 (7.4–13.1)
	3 months post-vaccination	583.6 (430.4–791.4)	1.4 (1.1–1.8)	732.3 (528.8–1,014.2)	1.2 (0.9–1.5)	280.9 (153.6–513.5)	2.4 (1.3–4.5)
BA.5	Pre-vaccination	93.7 (66.2–132.8)	Ref.	147.3 (103.8–209.1)	Ref.	28.5 (23.1–35.1)	Ref.
	1 month post-vaccination	626.9 (453.6–866.4)	6.5 (4.9–8.6)	922.9 (694.6–1,226.2)	6.3 (4.5–8.8)	204.3 (117.8–354.2)	7.3 (4.2–12.5)
	3 months post-vaccination	531.7 (367.8–768.6)	5.3 (3.9–7.2)	791.3 (560.8–1,116.5)	5.4 (3.7–7.8)	147.6 (84.5–257.9)	5.2 (2.8–9.7)
BN.1	Pre-vaccination	53.7 (41.6–69.3)	Ref.	74.2 (57.5–95.7)	Ref.	22.9 (18.0–29.2)	Ref.
	1 month post-vaccination	285.5 (204.8–398.0)	5.2 (4.1–6.5)	444.2 (336.0–587.2)	6.0 (4.6–7.9)	79.2 (53.4–117.6)	3.4 (2.6–4.5)
	3 months post-vaccination	213.3 (153.1–297.0)	3.8 (3.0–4.9)	308.0 (228.5–415.3)	4.2 (3.1–5.5)	65.2 (38.5–110.5)	3.0 (1.7–5.0)
XBB.1.5	Pre-vaccination	55.4 (46.7–65.7)	Ref.	63.4 (51.6–77.9)	Ref.	38.8 (31.4–47.9)	Ref.
	1 month post-vaccination	227.2 (176.5–292.6)	4.0 (3.2–5.2)	306.9 (240.3–392.0)	4.8 (3.6–6.4)	95.0 (72.4–124.7)	2.4 (1.6–3.6)
	3 months post-vaccination	171.7 (128.7–229.2)	3.0 (2.3–4.0)	225.5 (167.4–303.7)	3.6 (2.5–5.0)	71.4 (47.5–107.3)	1.8 (1.1–2.9)

*One individual contracted COVID-19 between the time of bivalent vaccination and one month later and another did so between one month and three months after vaccination. These individuals were excluded in further analysis i.e., 1-month post-vaccination GMT and GMFR were calculated using data from 39 individuals (10 for the infection-naïve group), and 3-month post-vaccination values were calculated using data from 38 individuals (nine for the infection-naïve group); †Among previously infected individuals, all but one reported being diagnosed with COVID-19 after February 14, 2023. The median (interquartile range) time from COVID-19 diagnosis and BA.4/5-containing bivalent vaccination was 8.5 (8–9) months.

against BA.5, that against BN.1 was 2.2-fold lower (95% CI for fold decrease, 1.8–2.6; p<0.001) and that against XBB.1.5 was 2.8-fold lower (95% CI, 2.2–3.4; p<0.001). Three months after, nAb titer against BN.1 was 2.5-fold lower (95% CI, 2.0–3.1; p<0.001), and that against XBB.1.5 was 3.1-fold lower (95% CI, 2.5–3.9; p<0.001), compared to that against BA.5. Phylogenetic analysis classified the viruses which were used in this study as Wuhan-like A, BA.5, BN.1, and XBB lineage. The reconstructed phylogenetic tree shows that XBB and BA.5 were not genetically clustered with each other (**Fig. 1B**).

When participants were divided into previously infected and infection-naïve groups, the pre-vaccination nAb titers against all examined variants were significantly higher in the previously infected group than in the infection-naïve group (**Fig. 2**). Intriguingly, while the previously infected group showed lesser GMFR than the infection-naïve group against the ancestral SARS-CoV-2 (GMFR one month after vaccination: 2.9 [95% CI, 2.3–3.6] vs. 9.8 [95% CI, 7.4–13.1], p<0.001), the GMFR was greater in the previously-infected group than the infection-naïve group against XBB.1.5 (GMFR one month after vaccination: 4.8 [95% CI, 3.6–6.4] vs. 2.4 [95% CI, 1.6–3.6], p=0.011). The highest observed nAb GMTs against BN.1 and XBB.1.5 were lower than that against the ancestral SARS-CoV-2 at baseline, although statistical significance was observed only in the previously infected group (data not shown). Two individuals who seroconverted to anti-N IgG positivity during the study period were excluded from these comparisons.

DISCUSSION

We found that the BA.4/5-containing bivalent mRNA vaccine significantly boosted nAb levels against both the ancestral SARS-CoV-2 and Omicron subvariants. Participants with a history of SARS-CoV-2 infection had higher nAb titers against all examined strains than the

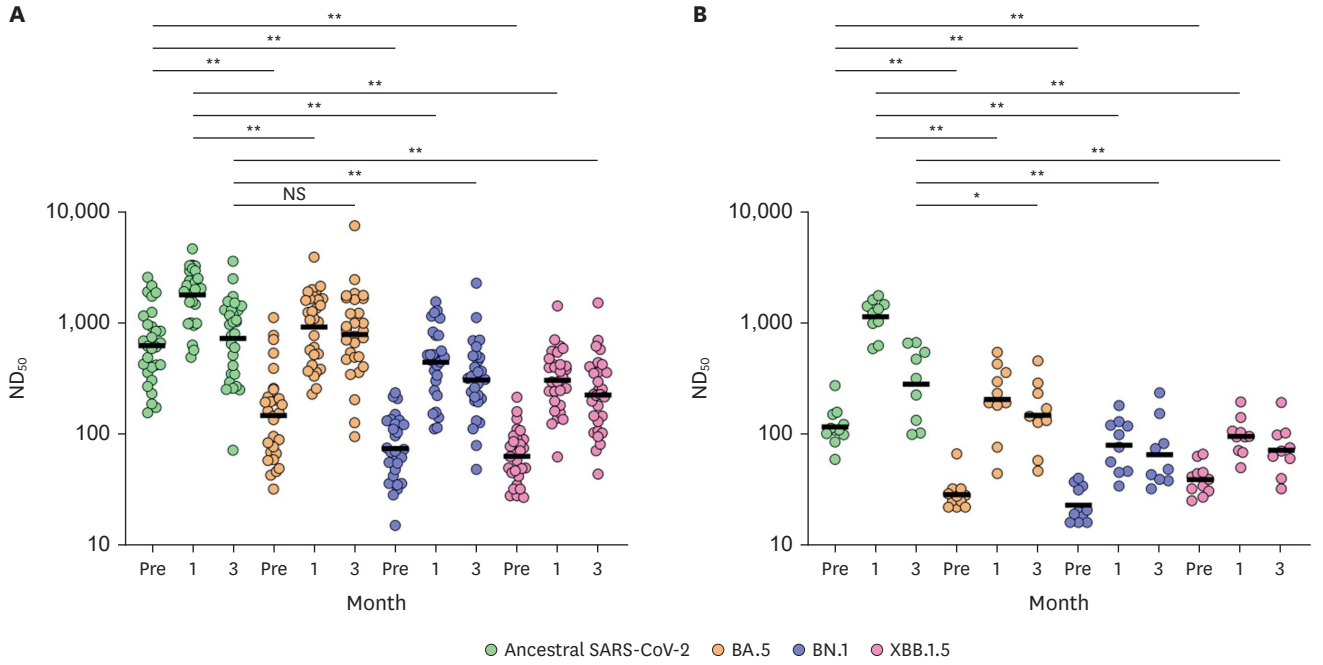

Figure 2. Neutralizing activities of the BA.4/5-containing bivalent mRNA vaccine-induced antibody to the Omicron subvariants in the individuals previously vaccinated with a monovalent booster dose. Neutralizing Ab titers against ancestral SARS-CoV-2, BA.5, BN.1, and XBB.1.5 at baseline, one month, and three months after BA.4/5-containing bivalent vaccination in the previously SARS-CoV-2 infected group (A) and infection-naïve group (B). The line indicates geometric mean titer of ND_{50} in each group.
NS, not significant.
*0.001≤p<0.01.
**p<0.001.

infection-naïve group. NAb titers against BN.1 and XBB.1.5, even at their highest observed values, were lower than those against the ancestral SARS-CoV-2 strain and BA.5 at the pre-vaccination time point.

BN.1, a sublineage derived from BA.2.75, was first identified in late July 2022 (14). Cao et al. reported that neutralizing ability of convalescent plasma of patients who had been infected with BA.5 after receiving three doses of Coronavac against BN.1 was 9.3-fold lower than against BA.4/5 (15). Liu et al. (16) collected blood samples from healthy volunteers 1 and 3 months after the third dose of BBIBP-CorV inactivated vaccine. Percentages of samples with detectable neutralizing activity against BN.1 (19%) were much lower than those against wild type (100%), BA.1 (88%), and BA.4/5 (81%).

XBB.1.5, a descendant of XBB, has been increasing since the fall of 2022 and is considered one of the most immune-evasive variants to date. Many studies on immune evasion by XBB have been reported (16-21). Several studies have demonstrated an approximately 20-fold decrease in the neutralization capacity of BA.5-convalescent plasma against XBB.1 compared to that against BA.4/5 (15,18,19). Davis-Gardner et al. (21) also showed that post-BA.4/5-containing bivalent vaccination nAb GMT against XBB was 25.8 times lower than that against the ancestral SARS-CoV-2, compared to a 4.3-fold difference for BA.5.

Individuals with previous SARS-CoV-2 infection had higher nAb titers against BA.4/5, BN.1, and XBB.1.5 compared to those without. Among the 29 individuals with a previous SARS-CoV-2 infection, the timing of SARS-CoV-2 infection was confirmed in 28. Twenty-five (89%)

individuals contracted COVID-19 between February 2022 and May 2022, when BA.1 and BA.2 were predominant in Korea (22). However, nAb titers against BN.1 and XBB.1.5 were lower than those against the ancestral SARS-CoV-2 and BA.5, even in individuals with recent infections with earlier Omicron subvariants. This is compatible with previous findings that BN.1 and XBB.1.5 evade the immunity induced by BA.4/5 (15,21,23). Therefore, vaccination with updated boosters would be necessary to ensure adequate protection against infection from newly emerging immune-evading variants.

In our study, the decrease in nAb titers from 1 month to 3 months after bivalent COVID-19 vaccination appears to be more marked for the ancestral SARS-CoV-2 strain compared to other variants. All of our study subjects received a primary series and booster dose of the COVID-19 vaccine containing the ancestral SARS-CoV-2 spike. Therefore, immune imprinting by the exposure to the ancestral SARS-CoV-2 through the previous COVID-19 vaccination might affect the high nAb titer against the ancestral SARS-CoV-2 strain after the BA.4/5-containing bivalent mRNA vaccination. There is a lack of information on whether there are differences in the kinetics of waning Ab according to the SARS-CoV-2 strains and the titers. The extent of Ab waning against the ancestral SARS-CoV-2 seems to be similar or lesser than that against BA.2 and BA.5 after bivalent mRNA vaccination (24,25). However, in the study conducted by Branche et al., (26) the uninfected individuals who were boosted with the Pfizer BA.1 + wild-type vaccine showed a larger geometric mean fold decline estimate for wild-type strain than for BA.1 from day 29 to day 91, and this is consistent with our findings. In addition, because a rate of Ab decay slows over time (27), there is a possibility that the differences of slope of waning titers between different variants might become smaller if follow-up period was longer. Further studies on the kinetics of Ab against different SARS-CoV-2 strains would be required.

Our study has several limitations. First, our findings may not be generalizable because the sample size was small and the study population consisted mainly of young women. In addition, the duration for the observation of nAb activities is limited to three months after the bivalent vaccination. Second, we cannot guarantee that the participants who were infected with SARS-CoV-2 during the Omicron predominant period were infected with the Omicron variant because of the lack of sequencing data. However, considering that all but one person was infected after February 14, 2022, when the Omicron variant accounted for more than 99% of SARS-CoV-2 infection cases in Korea, it may be reasonable to assume that these people were infected with the Omicron variant. Third, the absence of a self-reported COVID-19 history and anti-N IgG negativity does not necessarily mean that the person is infection-naïve because anti-N IgG levels could wane. Fourth, we did not investigate the T cell response, which is a crucial immune component in preventing severe disease. A potent T cell response against XBB among bivalent vaccine recipients was demonstrated, and the conservation of T cell epitopes in Omicron variants has been reported (28-30).

In conclusion, the BA.4/5-containing bivalent mRNA vaccine induced lesser nAb against later Omicron subvariants, especially XBB.1.5, than against ancestral SARS-CoV-2 and BA.5. To achieve better protection, vaccination with updated vaccines would be desirable, especially for populations at high risk of severe COVID-19.

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