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Persistence of immunity against Omicron BA.1 and BA.2 variants following homologous and heterologous COVID-19 booster vaccines in healthy adults after a two-dose AZD1222 vaccination

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ABSTRACT

Objectives: The SARS-CoV-2 Omicron variant presents numerous mutations potentially able to evade neutralizing antibodies (NAbs) elicited by COVID-19 vaccines. Therefore, this study aimed to provide evidence on a heterologous booster strategy to overcome the waning immunity against Omicron variants.
Methods: Participants who completed the Oxford/AstraZeneca (hereafter AZD1222) vaccine dose for 5-7 months were enrolled. The reactogenicity and persistence of immunogenicity in both humoral and cellular response after a homologous or heterologous booster with the AZD1222 and messenger RNA (mRNA) vaccines (BNT162b2, full, or half-dose mRNA-1273) administered 6 months after primary vaccination were determined.
Results: A total of 229 individuals enrolled, and waning of immunity was observed 5-7 months after the AZD1222-primed vaccinations. Total receptor-binding domain (RBD) immunoglobulin (Ig) levels, anti-RBD IgG, and focus reduction neutralization test against Omicron BA.1 and BA.2 variants and T cell response peaked at 14-28 days after booster vaccination. Both the full and half dose of mRNA-1273 induced the highest response, followed by BNT162b2 and AZD1222. At 90 days, the persistence of immunogenicity was observed among all mRNA-boosted individuals. Adverse events were acceptable for all vaccines.
Conclusion: A heterologous mRNA booster provided a significantly superior boost of binding and NAbs levels against the Omicron variant compared with a homologous booster in individuals with AZD1222-primed vaccinations.

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Introduction

Since the first emergence of the SARS-CoV-2 Omicron (BA.1/B.1.1.529) variant in November 2021, it has rapidly spread and become the dominant variant circulating worldwide (World Health Organization, 2022a; World Health Organization HQ, 2022). The Omicron variant harbors mutations within the Spike (S) protein, particularly 15 amino acid substitutions in the receptor-binding domain (RBD) (Viana et al., 2022). Mutations within the RBD of the Omicron variant mediate antibody evasion and greatly increase transmissibility through enhanced affinity for the angiotensin-converting enzyme 2 receptor (ACE2) (Mannar et al., 2022; McCallum et al., 2022; Tian et al., 2021). Recently, the Omicron variant was further classified into several descendant sublineages, including BA.1, BA.1.1, BA.2, BA.2.2, and BA.3. (Viana et al., 2022). As of March 2022, epidemiological data have suggested that BA.2 has been the most common sublineage of Omicron worldwide, including in the South-East Asia region (World Health Organization, 2022b). Furthermore, as in Thailand, epidemiological surveillance revealed that the proportion of the BA.2 variant has increased and represented >90% of all positive cases reported since March 2022 (Puenpa et al., 2022). BA.1 and BA.2 share multiple mutations, however, BA.2 presents unique viral characteristics to BA.1, such as a higher reproduction rate, fusogenicity, and pathogenicity (Viana et al., 2022; Yamasoba et al., 2022). However, scientific knowledge on the difference in ability between BA.1 and BA.2 to evade third-dose vaccine-induced immunity is currently limited.

Several COVID-19 vaccines have been developed to combat the SARS-CoV-2 infection. The AZD1222 vaccine has been the highly used vaccine accounting for 2.8 billion doses administered worldwide and 48.3 million doses in Thailand alone (AstraZeneca, 2022)(Department of Disease Control 2022). Although the vaccine effectiveness (VE) after two-dose AZD1222 was estimated at 64.0-74.0% for preventing SARS-CoV-2 and other lineage infections (Clemens et al., 2021; Falsey et al., 2021), the waning of vaccine-induced immunity of both anti-immunoglobulin (Ig) G and the neutralizing antibodies (NAbs) from AZD1222-primed vaccinees has been documented. For example, the dramatically decreased levels of NAbs after 5-6 months of two doses of AZD1222 vaccination showed an inadequate response to control the spread of SARS-CoV-2, especially the Omicron variant (Dejnirattisai et al., 2022; Planas et al., 2022; Shrotri et al., 2021). Similarly, NAbs decreased substantially after 6 months of vaccination with BNT162b2 (Dejnirattisai et al., 2022). Furthermore, the increase in infection was related to the waning immunity as a function of time after BNT162b2-priming (Goldberg et al., 2021; Levin et al., 2021). Thus, the decline in vaccine-induced immunity markedly increased a few months after vaccination and may be the potential cause of breakthrough infection.

There is a growing interest in using an additional booster dose as a new strategy to combat waning immunity after primary vaccination and the high transmissibility and immune evasion of the Omicron sublineages. To date, increasing evidence has supported that the booster dose of the messenger RNA (mRNA) vaccine following BNT162b2-primed vaccine significantly increased protection against the Omicron variant (Garcia-Beltran et al., 2022). Furthermore, a heterologous booster after 6 months of two-dose CoronaVac produced stronger humoral and cellular immunity than a homologous booster (Assawakosri et al., 2022). In individuals primed with AZD1222, a heterologous boost with BNT162b2 exhibited more effectiveness in inducing NAbs against BA.1 than the homologous booster with AZD1222 (Dejnirattisai et al., 2022); however, there were no data against BA.2. These results implied that the heterologous booster strategy could provide stronger immunity against Omicron infection. Nonetheless, minimal knowledge is available on the duration of immune protection after the third homologous or heterologous dose and when we should consider an additional booster beyond the third dose.

Accordingly, our objective was to study the persistence of immunogenicity beyond 1 month following the third dose. We also investigated the NAb against the emerging Omicron variant, particularly the BA.2 sublineage, the cellular response, and the reactogenicity after the homologous and heterologous booster dose administered at 6-month intervals in healthy adults who were previously vaccinated with AZD1222.

Materials and methods

Study design

This prospective cohort study was conducted at the Clinical Trial Unit, the Center of Excellence in Clinical Virology of Chulalongkorn University, Bangkok, Thailand, and was performed in accordance with the principles of the Declaration of Helsinki and the Good Clinical Practice Guidelines. The participants were informed, and written consent was obtained before enrollment.

Study participants

Between November 2021 and January 2022, a total of 229 subjects participated in this prospective cohort study. The main inclusion criteria were as follows: Thai adults aged >18 years with no previous or current diagnosis of COVID-19 infection and who completed a two-dose vaccination of the AZD1222 at an interval of 5-7 months during the enrollment period. The intervals between two-dose AZD1222-primed were 8-10 weeks. In addition, participants with an autoimmune disease or cancer, taking immunosuppressive drugs, or pregnant or breastfeeding participants were excluded.

Study vaccine

All participants were divided into four groups by conveniently sampling 50-60 participants per group based on vaccine availability without randomization. Participants were vaccinated with one of the following preparations; including viral vector: AZD1222 (AstraZeneca, Oxford, UK) (Folegatti et al., 2020), mRNA: BNT162b2 (Pfizer-BioNTech Inc., New York City, New York), mRNA: 100 μg mRNA-1273 (full-dose group) (Moderna Inc., Cambridge, Massachusetts), and 50 μg mRNA-1273 (half-dose group) (Jackson et al., 2020). The study flow is illustrated in Figure 1.

Reactogenicity assessment

All participants were observed under the supervision of doctors after vaccination to prevent anaphylaxis reactions. Participants received an online or paper-based self-monitoring record survey 7 days after vaccination. They observed the solicited local and systemic adverse events (AEs), including injection site pain, induration, redness, fever, headache, myalgia, arthralgia, nausea, and vomiting.

Sample collection, total RBD Ig, and anti-RBD IgG/nucleocapsid assay

Blood samples were collected before vaccination and at every follow-up visit on days 14, 28, and 90 after the booster dose. The samples of all participants were tested for quantitative antibody

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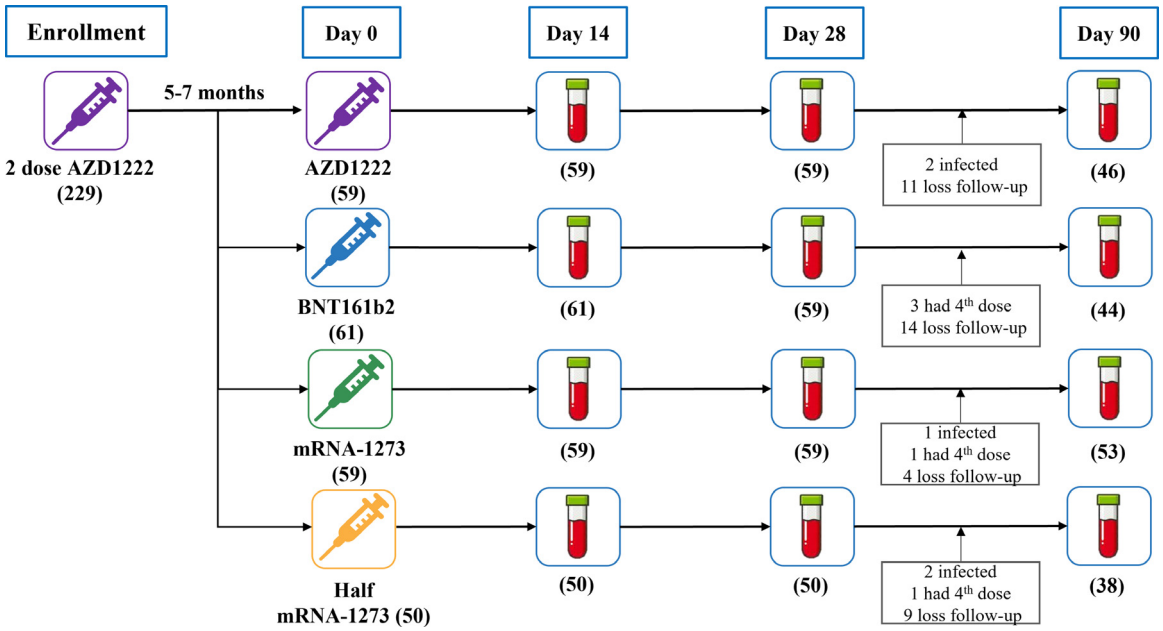

Figure 1. Diagram of the number of included participants and study groups
A total of 229 individuals who were previously vaccinated with AZD1222 were enrolled to analyze binding antibodies and T cell responses in the cohort study. Blood samples were collected at 0, 14, 28, and 90 days after booster vaccination. They were assigned to receive a booster vaccine, including AZD1222 (n = 59), or BNT162b2 (n = 61), or mRNA-1273 (n = 59), or half-dose mRNA-1273 (n = 50). mRNA = messenger RNA.

levels of total RBD Ig using Elecsys SARS-CoV-2 S (Roche Diagnostics, Basel, Switzerland), anti-RBD IgG, and anti-nucleocapsid IgG (anti-N IgG) using the SARS-CoV-2 IgG assay (Abbott, Sligo, Ireland) (Kanokudom et al., 2022).

Surrogate virus neutralization assay for Delta and Omicron variants

A cPass™ SARS-CoV-2 surrogate virus neutralization test (sVNT) (GenScript Biotech, New Jersey) was used to measure NAb titers against SARS-CoV-2 variants. Recombinant RBD from Delta and Omicron strains and 96-well plates coated with recombinant human ACE2 was used as previously described (Kanokudom et al., 2022). Samples with percent inhibition ≥30% were considered "seropositive" for SARS-CoV-2 NAbs.

Focus reduction neutralization test against the BA.1 and BA.2 variants

The neutralization antibody titers against the variants BA.1 and BA.2 of SARS-CoV-2 Omicron were measured. The focus reduction neutralization test (FRNT50) assay was performed as previously described (Assawakosri et al., 2022). The nucleotide sequences of the BA.1 and BA.2 variants are deposited in GenBank under accession numbers: EPI_ISL_8547017 for BA.1 and EPI_ISL_11698090 for BA.2 variant. The 50% focus reduction was calculated, and the 50% inhibitory concentration was determined using PROBIT regression analysis (SPSS Inc., Chicago, Illinois, USA). The detection limit of the assay is 1:20, and NAb values below the limit were substituted with a titer of 10.

Interferon-gamma release assay

The interferon-gamma (IFN-γ) release assay was performed to evaluate cell-mediated immunity using QuantiFERON SARS-CoV-2 research use only (QIAGEN, Hilden, Germany) (Kanokudom et al., 2022). Briefly, whole blood was added to an S-peptides-coated tube (Ag1) to stimulate clusters of differentiation (CDs) CD4+ and (Ag2) to stimulate CD4+/CD8+ T cells, then incubated for 21 hours. Plasma samples were then collected to measure IFN-γ levels by enzyme-linked immunosorbent assay. The IFN-γ levels were expressed in the international unit (IU) IFN-γ/ml. Levels above 0.15 IU/ml were defined as seropositive.

Statistical analysis

G*Power software version 3.1.9.6 was used to calculate the sample size. All statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS) version 22.0 (SPSS Inc., Chicago, Illinois, USA). Figures were generated using GraphPad Prism version 9.4.0 (GraphPad Software, San Diego, California, USA) and R version 4.1.2. Software (R Foundation for Statistical Computing, Vienna, Austria). Binding antibody and NAbs were logarithmically transformed, and comparison between groups was performed using analysis of variance with Bonferroni adjustment or Kruskal-Wallis H test for nonparametric data. The qualitative data comparison was performed using Pearson's χ^2 or Fisher's exact test. The Wilcoxon signed-rank test was used for pairwise analysis. A *P*-value <0.05 was considered statistically significant.

Results

Demographic data

From November 2021 to January 2022, 229 adults were enrolled in the study. All participants were healthy Thai adults with a mean age of 50.05 (± 9.71) years, 55.72 (± 13.68) years, 50.66 (± 12.95) years, and 57.02 (± 12.28) years in group AZD1222, BNT162b2, full-dose mRNA-1273, and half-dose mRNA-1273, respectively. Most participants (55.5%) were female. The average interval between the second dose and the booster dose was 166.3 (128-229) days. Common comorbidities included dyslipidemia, hypertension, diabetes mellitus, and allergy. There was no significant difference in baseline characteristics, including sex, common underlying diseases, and the intervals between follow-up times among all groups. However, the age of BNT162b2 and half-dose mRNA-1273 recipients was significantly higher than that

Table 1
Baseline characteristics of the participants enrolled in this study

	Total	AZD1222	BNT162b2	mRNA-1273	Half mRNA-1273
Total number (n)	229	59	61	59	50
Age ± SD (year)					
Mean ± SD	53.24 ± 12.55	50.05 ± 9.71	55.72 ± 13.68	50.66 ± 12.95	57.02 ± 12.28
Sex					
Male (%)	102 (44.5%)	23 (39.0%)	26 (42.6%)	30 (50.8%)	23 (46.0%)
Female (%)	127 (55.5%)	36 (61.0%)	35 (57.4%)	29 (49.2%)	27 (54.0%)
Underlying diseases (%)					
Allergy	14 (6.1%)	5 (8.5%)	5 (8.2%)	3 (5.1%)	1 (2.0%)
Cardiovascular diseases	4 (1.7%)	3 (5.1%)	1 (1.6%)	0 (0.0%)	0 (0.0%)
Diabetes mellitus	19 (8.3%)	2 (3.4%)	8 (13.1%)	5 (8.5%)	4 (8.0%)
Dyslipidemia	46 (20.1%)	6 (10.2%)	17 (27.9%)	10 (16.9%)	13 (26.0%)
Hypertension	46 (20.1%)	9 (15.3%)	17 (27.9%)	7 (11.9%)	13 (26.0%)
Thyroid	4 (1.7%)	1 (1.7%)	3 (4.9%)	0 (0.0%)	0 (0.0%)
Others	20 (8.7%)	7 (11.9%)	10 (16.4%)	3 (5.1%)	0 (0.0%)
Interval between second and booster dose Mean (range) day	166.3 (91-229)	155.5 (149-208)	171.4 (145-229)	169.4 (155-175)	168.5 (91-206)
Follow-up time					
Second visit					
Mean (range) day	14.7 (12-20)	14.6 (14-20)	16.0 (14-19)	14.1 (13-15)	14.0 (12-21)
Third visit					
Mean (range) day	29.9 (21-39)	28.9 (28-36)	29.9 (26-39)	28.2 (26-30)	33.2 (21-35)
Fourth visit					
Mean (range) day	94.5 (89-114)	90.3 (90-92)	102.8 (93-114)	91.8 (89-100)	94.4 (90-104)

mRNA= messenger RNA; SD = standard deviation.

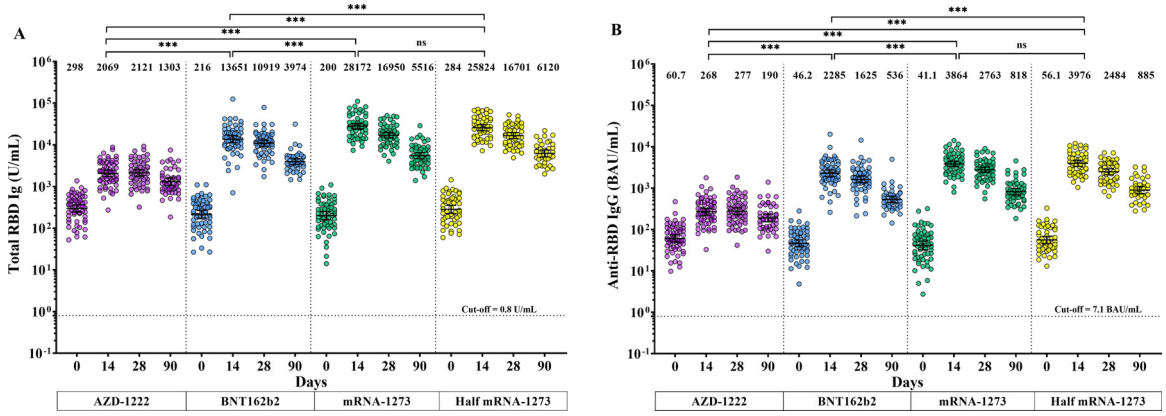

Figure 2. Measurement of the SARS-CoV-2-specific binding antibody
(A) Shows the total RBD Ig (U/ml). (B) Displays the anti-RBD IgG (BAU/ml). Each data point represents the level of SARS-CoV-2-specific binding antibody in sera from an individual who completed two doses of AZD1222, followed by the booster vaccine, including AZD1222 (purple), BNT162b2 (blue), mRNA-1273 (green), or half-dose mRNA-1273 (yellow). The error bars indicate GMT and 95% CI. The dotted lines designate the cutoff values. ns indicates that there is no statistical difference; **, P <0.01, ***, P <0.001.
BAU = binding antibody units; CI = confidence interval; GMT = geometric mean titer; IgG = immunoglobulin G; mRNA = messenger RNA; RBD = receptor-binding domain.

of the AZD1222 and mRNA-1273 arms. Participant characteristics are listed in Table 1. Of the 229 subjects in this study, two subjects (BNT162b2 group) withdrew from the study before the day 28 blood sampling time point. There were 13 (AZD1222), 15 (BNT162b2), 6 (mRNA-1273), and 12 (half-dose mRNA-1273) subjects that did not complete the visit at the day 90 blood sampling time point. The most common reason was loss to follow-up.

Immunogenicity assessment

Total RBD Ig and anti-RBD IgG

There were no differences in total RBD Ig and anti-RBD IgG among the four groups at baseline. Most participants had a very low antibody titer 5-7 months after completing the second AZD1222 vaccination. However, 28 days after the booster, the total RBD Ig levels were significantly elevated (Figure 2a). Using the homologous (AZD1222) group as a reference group, the geometric mean ratio (GMR) ranged from 4.68 (95% CI: 3.42-6.40) in the BNT162b2 to 7.91 (95% CI: 5.86-10.67) in the mRNA-1273 groups. Comparable trends with anti-RBD IgG levels were observed (Figure 2b). The GMRs ranged from 5.43 (95% CI: 3.92-7.52) in the BNT162b2 to 10.14 (95% CI: 7.41-13.83) in the mRNA-1273 groups (Supplementary Table 1). At 90 days after vaccination, a significant decrease in total RBD Ig and anti-RBD IgG was observed. The reduction percentage ranged between 30-70% depending on booster vaccines (Supplementary Table 2). Overall, the mRNA-1273 vaccine produced the highest immune response among all booster groups, followed by half mRNA-1273, BNT162b2, and AZD1222, respectively. The geometric mean titer (GMT) of total RBD Ig and anti-RBD IgG between full- and half-dose mRNA-1273 were comparable at both 14 and 90 days. Anti-N IgG levels were also measured. In this study, the anti-N IgG results showed that all participants were seronegative (cutoff 1.4) to anti-N IgG at each visit (Supplementary Figure 1).

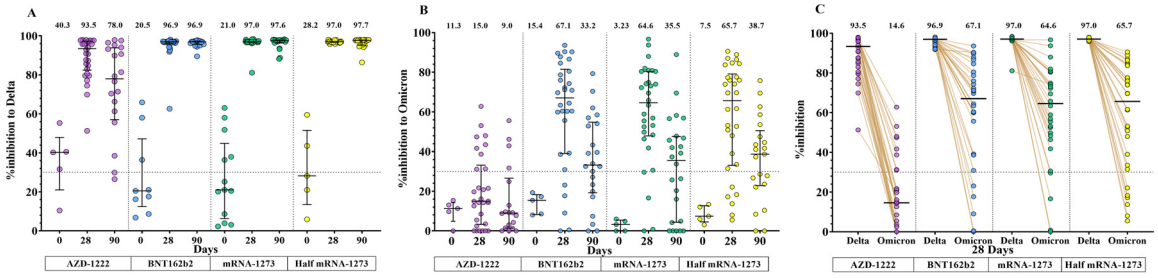

Figure 3. Neutralizing activities of Delta and Omicron variants compared between preboost and postboost using a surrogate virus neutralization test
A subset of samples was randomly selected to test for sVNT, including sera collected at baseline and sera collected 28 days and 90 days after boost (n = 30/group). (A) Neutralizing activities against the SARS-CoV-2 Delta variant and (B) neutralizing activities against the SARS-CoV-2 Omicron variant (BA.1) were shown as % inhibition. (C) Comparison of the neutralizing activity between the Delta and Omicron variants after booster vaccination. Median values with IQR are denoted as horizontal bars. Horizontal bars indicate the median. Dotted lines designate cutoff values (30%).
IQR = interquartile range; mRNA = messenger RNA; sVNT = surrogate virus neutralization test.

Surrogate virus neutralization specific Delta and Omicron variants

A subset of the sample was tested for sVNT in the prebooster stage. At 28 days after boosters, the median percentage inhibition (% inhibition) to the Delta variant was significantly elevated to 93.5% in AZD1222, 96.9% in BNT162b2, 97.0% in mRNA-1273, and 97.9% in half-dose mRNA-1273 (Figure 3a). Whereas, a lower % inhibition was observed against Omicron variants with 15.0%, 67.1%, 64.6%, and 65.7% in groups AZD1222, BNT162b2, mRNA-1273, and half-dose mRNA-1273, respectively (Figure 3b). These findings suggested that heterologous-boosted individuals achieved a higher level of NAbs against the Omicron variant than homologous-boosted individuals. On day 90 after vaccination, the median % inhibition to Delta variant slightly decreased to 78.0% in the AZD1222-boosted group, while the other groups remained >97% inhibition. In contrast to the Omicron variant, the median % inhibition was reduced to 9.0% for AZD1222, 33.2% for BNT162b2, 35.5% for mRNA-1273, and 38.7% for half-dose mRNA-1273, respectively. Correspondingly, the neutralizing activity against the Omicron variant was significantly lower than that against the Delta variant (Figure 3c). The heterologous AZD1222/mRNA prime-boosted achieved higher detectable neutralization activities against Omicron than the homologous booster at both the day 28 and day 90 time point (Supplementary Table 3).

Focus reduction neutralization test against the BA.1 and BA.2 variants

Most participants showed undetectable NAbs at baseline. Then, 28 days after receiving a booster, the GMTs of NAbs against BA.1 and BA.2 were significantly increased compared with baseline (Figure 4a and b). Consistent with those binding antibodies, the GMR of NAbs against BA.1 ranged from 5.56 (95% CI: 2.70-11.43) in the BNT162b2 group to 18.03 (95% CI: 8.89-36.56) in the mRNA-1273 group. While the GMR of NAbs against BA.2 ranged from 5.43 (95% CI: 2.64-11.17) in the BNT162b2 group to 7.11 (95% CI: 3.45-14.62) in the mRNA-1273 groups (Supplementary Table 1). Then, 90 days after vaccination, a significant decrease in NAbs levels was observed, and the percentage of reduction ranged between 30-75% depending on booster vaccines (Supplementary Table 2). The NAb titers of BA.1 were comparable with BA.2 (Figure 4c and d), and higher activity was observed to neutralize the Omicron variant in the heterologous mRNA-boosted than in the homologous AZD1222-boosted individuals.

Total interferon-gamma response

The IFN-γ release assay was used to measure the presence of T cell responses. At the baseline visit, the total IFN-γ level produced by CD4+ and CD4+/CD8+ was observed to be less in all participants, which showed the seropositivity rate of 25.0% and 37.1%, respectively. Conversely, the seropositivity rate for CD4+ rapidly increased to 79.3% for BNT162b2, 89.3% for mRNA-1273, and 89.3% for the half-dose group on day 14, whereas the seropositivity rate for CD4+/CD8+ were 86.2%, 89.3%, and 92.9%, respectively. Then it slightly decreased at 28 days after the booster (Figure 5a and 5b). The highest seropositivity rates in the AZD1222 group were 37.9% and 69.0%, indicating that AZD1222-boosted individuals produced a lower T cell response than the mRNA-boosted individuals.

Moreover, the persistence of T cell response was observed among mRNA-boosted individuals on day 90 after vaccination, but not in AZD1222-boosted individuals. Consequently, the heterologous mRNA booster exhibited a stronger positive impact on T cell responses than the homologous booster in individuals previously vaccinated with AZD1222. The levels of IFN-γ among all groups are listed in Supplementary Table 4.

Reactogenicity assessment

Reactogenicities following the booster vaccination were as follows: the most common local reaction in the first 7 days was injection site pain, whereas the most common systemic reaction was myalgia (Figures 6a-d). A comparison of AEs between groups revealed that the injection site pain, myalgia, and chills were lower in individuals receiving AZD1222 than in other vaccines (Supplementary Figure 2a-c). In addition, the incidences of injection site pain, swelling, fever, myalgia, joint pain, and chills in the mRNA-1273 group were significantly higher than those observed in the AZD1222 group (Supplementary Table 5). However, most incidences of AEs were reported as mild to moderate in severity and resolved within a few days after vaccination. Furthermore, the local reactions AEs were less frequent in half-dose mRNA-1273 than in full dose, including injection site pain (98.3% vs 86.0%) and swelling (50.8% vs 30.0%). During the study period, no serious AEs were observed among all groups.

Discussion

In this study, we report quantifications of total RBD Ig, anti-RBD IgG, NAb, and total IFN-γ levels after homologous and heterologous boosters in participants who received two doses of AZD1222 vaccine. We also show the reactogenicity profile of all vaccine boosters. There was a significant increase in total RBD Ig, anti-RBD IgG, NAb, and cellular responses at 14-28 days after the booster shot. The decay of the immune response was observed 90 days after the initial booster for all study arms. At 7 days after vaccination, all COVID-19 vaccines administered as a booster dose had acceptable reactogenicity with mild to moderate AEs and generally resolved within a few days after vaccination. AEs data indicated that homologous and heterologous vaccine boosters presented an acceptable safety profile in all healthy adults, consistent with the previous study of homologous and heterologous boost-

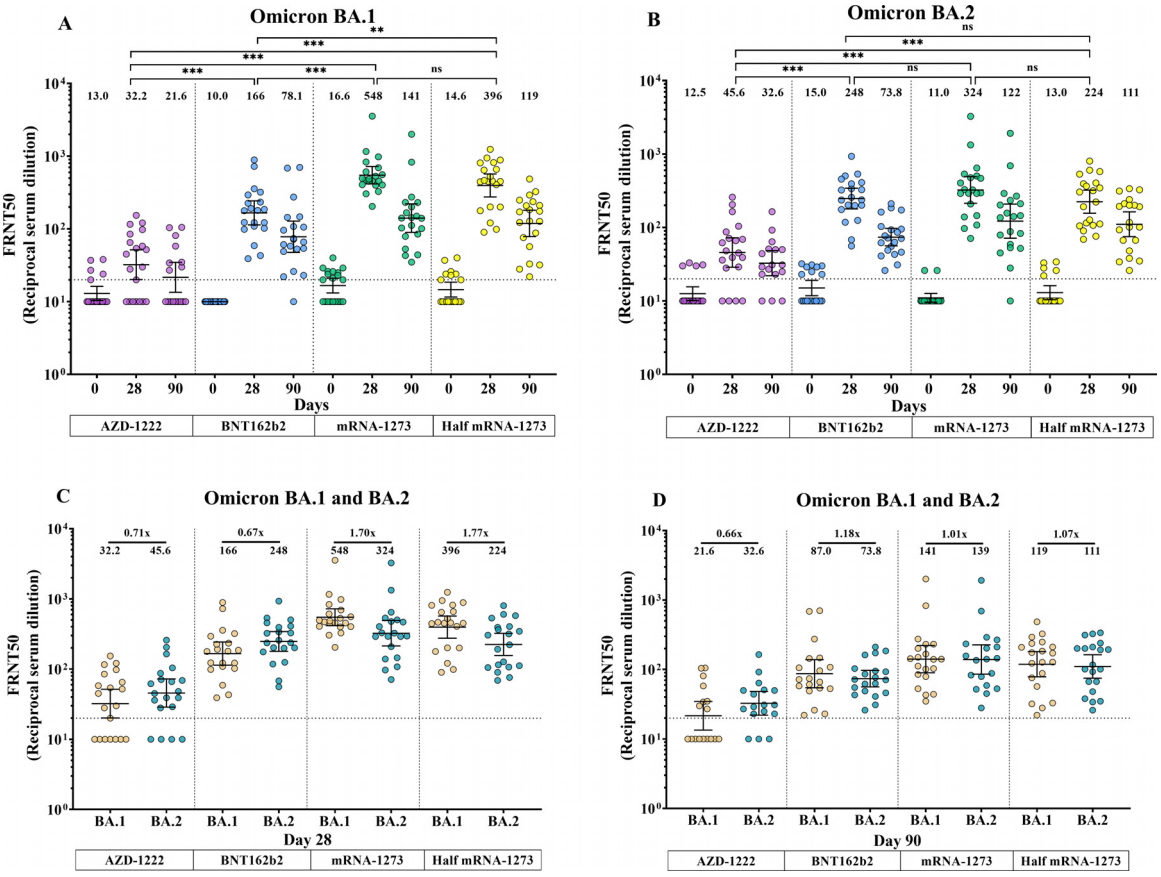

Figure 4. Neutralizing antibody titers against Omicron BA.1 and BA.2 before and after booster vaccination
Neutralization of SARS-CoV-2 Omicron BA.1 and BA.2 was measured using FRNT50. (A) Neutralization against the BA.1 Omicron variant and (B) neutralization against the BA.2 Omicron variant. (C) Comparison of NAb titers against BA.1 and BA.2 28 days after the booster dose. (D) Comparison of NAb titers against BA.1 and BA.2 90 days after the booster dose. Each data point represents an individual who received a booster vaccine, including the viral vector vaccine, AZD1222 (purple), the mRNA vaccine, BNT162b2 (blue), mRNA-1273 (green), or half-dose mRNA-1273 (yellow). The error bars present GMT and the 95% CI. Values below the limit were substituted with a titer of 10. The dotted lines designate the cutoff values (1:20).
ns indicates that there is no statistical difference; **, P <0.01, ***, P <0.001.
CI = confidence interval; FRNT50 = 50% focus reduction neutralization test; GMT = geometric mean titer; mRNA = messenger RNA; NAb = neutralizing antibodies.

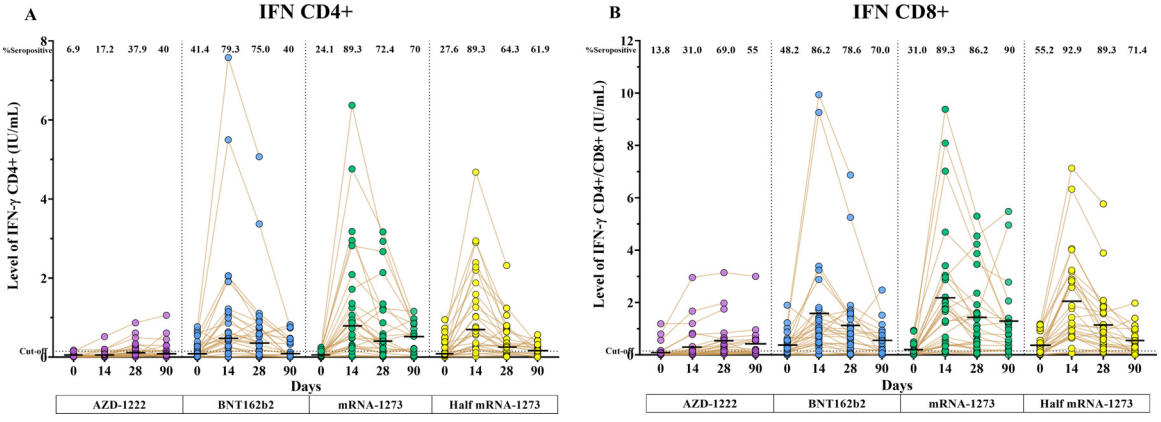

Figure 5. Total CD4+ and CD8+ T cell-producing IFN-γ assay
The QFN IFN-γ ELISA measured levels on days 0, 14, 28, and 90. (A) Shows the IFN-γ produced by CD4+ (stimulated with CD4+specific epitope RBD from RBD [Ag1]), and (B) shows the IFN-γ produced by CD4+ and CD8+ T cell (stimulated with CD4+/CD8+ specific epitope derived from S1 and S2 subunits [Ag2]). Each data point represents an individual who received a booster vaccine, including the viral vector vaccine, AZD1222 (purple), the mRNA vaccine, BNT162b2 (blue), mRNA-1273 (green), or half-dose mRNA-1273 (yellow). Horizontal bars indicate the median. The dotted lines designate the cutoff values (0.15 IU/mL).
CD = clusters of differentiation; ELISA = enzyme-linked immunosorbent assay; IFN-γ = interferon-gamma; mRNA = messenger RNA; QFN = QuantiFERON; RBD = receptor-binding domain.

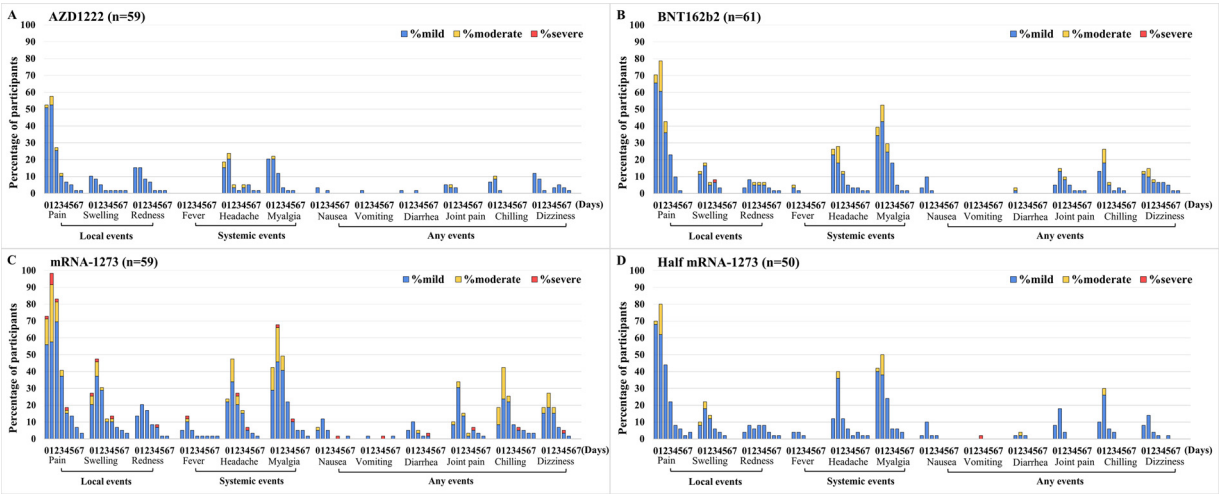

Figure 6. Reactogenicity and severity of local and systemic reactions during the first 7 days after vaccination with a booster dose of COVID-19 vaccines
The booster vaccine included the viral vector vaccine - AZD1222 (A), mRNA vaccine - BNT162b2 (B), mRNA vaccine - mRNA-1273 (C), and the half-dose mRNA-1273 (D). The percentage of participants who reported adverse events is presented on the Y-axis. Swelling and redness were classified by measuring the diameter area as mild (<5 cm), moderate (5 to <10 cm), and severe (≥10 cm). Fever was graded as mild (38°C to <38.5°C), moderate (38.5°C to <39°C), and severe (≥39°C). The other events were classified as mild (no impact on regular activity)/moderate (some limitation of daily activity), and severe (unable to perform or prevented daily activity).
mRNA = messenger RNA.

ers in individuals primed with the AZD-1222 or BNT162b2 vaccines (Flaxman et al., 2021; Munro et al., 2021).

Our findings revealed that after the first primary series of AZD1222, a waning immunity was evidenced 5-7 months after vaccination. After the booster was implemented with both homologous and heterologous vaccines, binding antibody levels increased significantly compared with baseline. Furthermore, individuals boosted with full and half doses of mRNA-1273 possessed the highest levels of total RBD Ig and anti-RBD IgG, followed by those boosted with BNT162b2 and AZD1222, respectively. Similar to the COV-BOOST study, the magnitude of anti-S IgG in individuals receiving a heterologous booster was significantly higher than the homologous booster (Munro et al., 2021). Moreover, half-dose mRNA-1273 elicited total RBD Ig and anti-RBD IgG responses equal to the full-dose group, which is consistent with the previous observation of mRNA-1273 booster in participants primed with two doses of mRNA-1273 (Choi et al., 2021). Therefore, the half-dose of mRNA-1273 could dramatically reduce costs and increase the supply of vaccine doses. Our results suggest that a single-shot heterologous booster could further enhance the immune response by stimulating the memory B cell response (Goel et al., 2021). In addition, a robust induction of B cells has been reported previously regarding the heterologous third dose in individuals receiving triple inactivated or triple mRNA vaccine (Gagne et al., 2022; Liu et al., 2022; Wang et al., 2022).

In accordance with those recent reports from the UK, we found that NAbs after 5-7 months of AZD1222-primed individuals rapidly waned to near or below the detection limit and were barely neutralized against Delta and Omicron variants (Wu et al., 2022). Herein, we observed that the heterologous mRNA-based booster, but not the AZD1222-boosted, effectively produced a high level of cross-neutralization against SARS-CoV-2 variants 28 days after the booster dose. The breadth and cross-reactivity of NAbs after the third dose may be related to increasing memory B cell affinity maturation through extensive somatic hypermutation similar to those observed in a previous mRNA-boosted study (Paschold et al., 2022). However, the reductions in neutralizing activity against Omicron variants were found compared with those against Delta variants in this study and also previous studies in BNT162b2-primed and CoronaVac-primed individuals (Assawakosri et al., 2022; Pérez-Then et al., 2022; Planas et al., 2022). Although the BA.2 sublineage was documented to have different viral characteristics than BA.1, our findings revealed that the neutralizing activity against the BA.1 and BA.2 variants was comparable. These results are consistent with previous studies indicating that BA.2 was only 1.4-fold lower in NAbs than the BA.1 variant after BNT62b2 booster in BNT162b2-primed and previously infected individuals (Chen et al., 2022; Yu et al., 2022). Our finding indicated that mRNA vaccines encoding the ancestral strain-S could induce a potent cross-NAb against Delta, Omicron, and both BA.1 and BA.2 subvariants.

NAbs are the leading correlation with protection against COVID-19 infection (Khoury et al., 2021). In our cohort, individuals boosted with mRNA possessed high levels of NAbs titer against BA.1 and BA.2 subvariants. The high NAb titers observed in this study were related to the increase in VE protection against symptomatic disease caused by the Omicron and Delta variants after mRNA booster vaccination among the AZD1222-primed individuals (Andrews et al., 2022). Although >90% of all participants had detectable NAb responses after 90 days of booster vaccination, the reduction in NAbs titers against the Omicron variant was evident. Similarly, VE data substantially decreased from 70.1% to 60.9% in mRNA-1273-boosted individuals and 62.4% to 39.6% in BNT162b2-boosted individuals in 10 or more weeks after the booster (Andrews et al., 2022). Since there is an evidenced benefit of a fourth dose given 7 months after the third dose (Munro et al., 2022), the result showed that the fourth dose elicited a high level of anti-S IgG in individuals receiving AZD1222primed plus BNT162b2 booster. The additional fourth dose should be optional for those who received the AZD1222 as the third dose because of the lower antibody response in this group.

In addition to humoral immune responses, vaccine-induced T cell responses have a potential role in cross-recognizing Alpha, Beta, Delta, and Omicron variants even if using a different vaccination regimen (Gao et al., 2022; Jarjour et al., 2021; Tarke et al., 2022). Because class I and II S epitopes were 70-80% conserved, it led to the preservation of CD4+ and CD8+ T cell responses (Tarke et al., 2022). Thus, the CD4+ and CD8+ T cells responses have remained stable primarily in frequency and magnitude against all variants of concern. Our study showed that CD4+ and CD8+ T cell-producing IFN-γ were rapidly restored af-

ter 14 days of the heterologous booster vaccination. In addition, >70% of the mRNA-boosted individuals demonstrated seropositive for T cell-producing IFN-γ response at 90 days after vaccination but showed only 50% seropositive for T cell-producing IFN-γ response in the AZD1222-boosted group. Beyond humoral responses, the heterologous booster could enhance the adaptive T cell response. The T cell response may be essential as a second-level cross-protection against other upcoming variants.

Potential limitations of our study may be attributed to the loss of some participants during follow-up. All booster vaccines were in multiple-dose vials, which needed to be administered in a short period. The time limit of keeping opened multi-dose vials made the randomization not feasible in our study. Moreover, the phenotype of CD4+ and CD8+ T cells was not examined after booster vaccination. Additional studies on the longevity of the humoral immunity over 6-12 months are needed.

In conclusion, the heterologous mRNA vaccine has an acceptable safety profile and could significantly enhance humoral and cellular responses after vaccination in AZD1222-primed individuals. In addition, the booster with full-dose and half-dose mRNA-1273 exhibited the highest humoral and cellular immune responses at 90 days after the booster dose, followed by BNT162b2 and AZD1222 vaccine. Receiving a heterologous booster dose could provide immune protection against new emerging SARS-CoV-2 variants.

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Ethics approval and consent to participate

The study protocol was approved by the Institutional Review Board of the Faculty of Medicine of Chulalongkorn University (IRB numbers 690/64). The study was registered with the Thai Clinical Trials Registry (TCTR 20210910002).

Declaration of Competing Interest

The authors have no competing interests to declare.

CRediT authorship contribution statement

Suvichada Assawakosri: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **Sitthichai Kanokudom:** Data curation, Formal analysis, Methodology. **Jira Chansaenroj:** Data curation. **Nungruthai Suntronwong:** Data curation, Formal analysis, Methodology. **Chompoonut Auphimai:** Methodology. **Pornjarim Nilyanimit:** Conceptualization. **Preeyaporn Vichaiwattana:** Methodology. **Thanunrat Thongmee:** Methodology. **Thaneeya Duangchinda:** Methodology. **Warangkana Chantima:** Methodology. **Pattarakul Pakchotanon:** Methodology. **Donchida Srimuan:** Data curation. **Thaksaporn Thatsanatorn:** Data curation. **Sirapa Klinfueng:** Methodology. **Natthinee Sudhinaraset:** Data curation. **Juthathip Mongkolsapaya:** Methodology. **Nasamon Wanlapakorn:** Conceptualization, Writing – review & editing. **Sittisak Honsawek:** Conceptualization, Writing – review & editing. **Yong Poovorawan:** Conceptualization, Project administration, Writing – review & editing.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2022.07.038.

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