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Safety and antibody responses of Omicron BA.4/5 bivalent booster vaccine among hybrid immunity with diverse vaccination histories: A cohort study

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ABSTRACT

This cohort study, conducted between July and August 2023, evaluated the adverse events (AEs) and immune response to a bivalent mRNA-1273.222 (containing sequences of the original Wuhan-H1 strain and the Omicron BA.4/5 variant) booster vaccine in 122 participants. The study included individuals with diverse vaccination histories, and their responses were assessed based on anti-receptor binding domain (RBD) IgG levels and neutralizing antibodies against the wild-type, Omicron BA.5, and XBB.1.16 variants. Following administration of the BA.4/5 bivalent vaccine, AEs were generally mild to moderate and well-tolerated within a few days. There were no reports of vomiting and no serious AEs or death. The findings demonstrated robust immune responses, with significant increases in anti-RBD IgG levels, particularly in groups that had received 3 –6 doses before the booster dose. The BA.4/5 bivalent booster effectively induced neutralizing antibodies against the vaccine strains, providing robust neutralization, including the XBB.1.16 strain. The study also highlighted that individuals with hybrid immunity, especially those assumed infected with the BA.5 strain or who had been infected twice, showed higher levels of robust neutralizing activity against Omicron XBB.1.16. Overall, these results indicate that the BA.4/5 bivalent booster vaccines can induce potent and good antibody responses in emerging Omicron sub-variants, supporting its efficacy as a booster in individuals with diverse vaccination histories.

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has resulted in over 700 million confirmed cases and 6.9 million deaths [1]. It can cause a flu-like illness called Coronavirus diseases-19 (COVID-19). In 2020, the first generation of COVID-19 vaccines was developed using the original strain of SARS-CoV-2, known as the ancestral Wuhan-H1 or wild type (WT). These vaccines were created using either the isolated or sequenced form of the virus. All the vaccines, including the whole inactivated vaccines made by Sinovac and Sinopharm, the viral vector vaccines made by Johnson & Johnson–Janssen and AstraZeneca, the purified protein vaccine made by

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Novavax, and the viral protein-encoded mRNA vaccines made by Pfizer and Moderna, were designed to prevent disease caused by the ancestral strain. Although various vaccines have been widely used in many countries, their administration has not completely controlled the spread of the disease due to the evolution of virus variants [2,3]. Breakthrough infections and reinfections with new variants are more frequently rising among both immunized and previously infected individuals [4,5].

The Omicron variant (B.1.1.529) outbreak was reported in November 2021 in South Africa, later known as subvariant BA.1. The ongoing emergence of new variants of Omicron has led to successive global waves of infection. The study has revealed that the Omicron variant features significant mutations in the spike protein, comprising over 30 mutations, including at least 15 mutations in the receptor binding domain (RBD) [6]. The RBD is a crucial component for viral entry and is targeted in vaccine development. Over the past few months to years, numerous genetically related subvariants have emerged and have quickly been supplanted by a new subvariant, including BA.2, BA.2.75, BA.3, BA.4/5, XBB, BQ, and so on [7]. There were many changes compared to the pre-omicron variants such as Alpha and Delta. This lineage displays a heightened capacity for infection and for evading the neutralizing antibodies produced by prior infections and vaccine-induced immunity [2,5]. Previous studies have shown that serum obtained from prior infected individuals and vaccinated individuals with original monovalent vaccines exhibited reduced neutralization efficacy against the Beta, Delta, and Omicron variants. Moreover, a notably diminished neutralization capacity against Omicron was observed in comparison to the pre-Omicron dominant strains [8–11]. Therefore, the new vaccination strategies related to the upcoming strains need to be addressed.

After the global spread of the Omicron lineage, it has emerged as a significant public health concern, posing a serious threat to the efficacy of existing COVID-19 vaccines and therapies. Utilizing the capability of mRNA technology to address variant strains, bivalent vaccines were developed to mitigate this emerging threat. On September 1, 2022, the bivalent vaccines developed by Moderna and Pfizer–BioNTech, targeting SARS-CoV-2, were introduced. These bivalent vaccines consist of equal amounts of spike protein-encoding messenger RNA from the ancestral strain and the omicron BA.4–BA.5 subvariants [12,13].

Previous studies showed that administering a booster dose with a BA.4/5 bivalent vaccine robustly enhanced neutralizing antibodies against both the WT and the Omicron BA.5, providing broad neutralization across the Omicron subvariants including BA, BQ, XBB, and CA sublineages [7,14,15]. The study in North Carolina, USA, indicated that those who had previously received vaccination or boosters with the BA.1 bivalent vaccines (Moderna and Pfizer–BioNTech) exhibited higher effectiveness against hospitalization or death caused by Omicron infection (subvariants BA.4.6, BA.5, BQ.1, and BQ.1.1) at 15–99 days compared to those who had received the original monovalent vaccine (61.8 % vs 24.9 %). Additionally, there was no difference in vaccine effectiveness between the Moderna and Pfizer–BioNTech BA.4/5 bivalent boosters [12]. Nationwide cohort analyses in Denmark, Finland, Norway, and Sweden demonstrated that administering a fourth dose of the BA.4/5 or BA.1 bivalent booster was associated with reduced rates of hospitalization and death related to Omicron infection (subvariants BA.5, BQ, BF, and XBB) in adults aged 50 years and older, compared to the three doses of original monovalent group Furthermore, there were no discernible differences in protection against Omicron between the BA.4/5 and BA.1 bivalent mRNA vaccines [16]. Many studies have indicated that bivalent mRNA booster vaccinations offer potential inhibition and effectiveness against symptomatic infection of the Omicron sublineage, ranging between 14 % and 52 % [17–19].

There is a diverse use of COVID-19 vaccines in countries that import vaccines, such as Thailand. Many studies of administering bivalent mRNA vaccines as boosters are predominantly conducted in populations that have received a primary regimen of mRNA vaccines. However, there remains uncertainty regarding the effectiveness of bivalent mRNA vaccines as booster doses in individuals who have received various mix-and-match vaccine regimens, a practice observed in many countries with imported vaccines. The objective was to assess adverse events following the booster dose of the Moderna bivalent mRNA-1273.222 vaccine (encoding the spike sequences of the original and BA.4/5 strains) in the individuals with various vaccine regimens. Additionally, we monitored antibody responses, including anti-RBD IgG levels, and neutralizing activities against the WT and the Omicron subvariants: BA.5 and XBB.1.16.

Material and methods

Study designs and participant enrollment

The cohort study was conducted between July and August 2023 at the Center for Excellence in Clinical Virology, Chulalongkorn University, Bangkok. This study protocol was approved by the Institutional Review Board (IRB) of the Faculty of Medicine of Chulalongkorn University (IRB 284/66) and was conducted following the principles of the Declaration of Helsinki. This trial was registered in the Thai Registry of Clinical Trials (TCTR 20210910002). Written informed consent was obtained from the participants before enrollment.

The inclusion criteria comprised healthy adults aged 18 and older who had received a minimum of two doses of a COVID-19 vaccine at least six months prior. Individuals with well-controlled comorbidities were also included. Individuals with a history of infection or those who had never been infected were permitted to participate in the study. Exclusion criteria comprised pregnant individuals and those with serious medical conditions, such as immunocompromised status, malignancies, and autoimmune diseases.

A total of 123 individuals were enrolled and initially screened by a physician and a trained nurse. All individuals completed a self-recorded questionnaire to provide information, including their sex, age, comorbidity, history of infection which confirmed by antigen test kits or real-time PCR, and vaccination records. Blood samples were collected before the administration of mRNA-1273.222 (day 0, baseline) and after the booster dose (day 28 $\pm$ 7). The serum samples were subjected to laboratory assessments. One individual was excluded from the study due to loss to follow-up.

Vaccine

The bivalent mRNA-1273.222 COVID-19 vaccine (Moderna Inc., Cambridge, MA), with an amount of 50 μg, contains 25 μg each of two mRNAs encoding the SARS-CoV-2 spike protein sequences of the original Wuhan-H1 strain (WA1/2020) and the Omicron BA.4/BA.5 variant (hereafter referred to as the BA.4/5 bivalent vaccine). A dose of vaccine was administered intramuscularly.

Safety assessments

The enrolled participants self-reported reactogenicity using a paper questionnaire, starting on the day of their initial vaccination and for 6 subsequent days (days 0–6). Local, systemic, and any adverse events (AEs) were classified as mild, moderate, and severe. Mild was defined as easily tolerated with no limitation on regular activity, moderate involved some limitation of daily activity, and severe meant being unable to perform regular daily activities. Fever was defined as mild: 38.0 °C to $<$ 38.5 °C; moderate: 38.5 °C to $<$ 39.0 °C; severe: $\geq$39.0 °C.

Laboratory assessments

Serum samples were collected to determine the anti-receptor binding domain of the SARS-CoV-2 spike protein IgG (anti-RBD IgG) using a chemiluminescent microparticle immunoassay (CMIA). The assay was performed on an Architect plus i1000SR, following the guidelines

provided by the manufacturer (Abbott Laboratories, Abbott Park, IL). The results were reported in geometric mean titer (GMT) in binding antibody units per milliliter (BAU/mL). Levels of anti-RBD IgG below 7.1 BAU/mL were categorized as negative. Additionally, the serum was used to examine the previous infection through screening of the total immunoglobulin anti-nucleoprotein of the SARS-CoV-2 (anti-N Ig) using Elecsys® Anti-SARS-CoV-2 N ECLIA (Roche Diagnostics GmbH, Mannheim, Germany). The assay was conducted using the Cobas e 411 system, following the manufacturer's instructions. The seropositive of anti-N Ig (hybrid immunity) was determined when the results were equal to or greater than 1.0 COI, whereas results below 1.0 COI were considered negative (vaccine alone).

A subgroup of serum samples was randomly selected based on infection history and anti-N Ig serostatus to assess the neutralizing activity against the WT (GenBank accession no. MN908947.3) and the Omicron BA.5 and XBB.1.16 strains (GISAIDs accession no. EPI_ISL_17646230 and EPI_ISL_19145913, respectively) using a live virus focus reduction neutralization test at 50 percent ($FRNT_{50}$). Briefly, heat-inactivated sera were utilized to create serial dilutions ranging from 1:10 to 1:7290, followed by incubation with a live virus for 1 h at 37 °C. The mixtures were then transferred to monolayers of Vero cells in a 96-well plate and incubated for 2 h. The foci development was examined and calculated as previously described [20]. The $FRNT_{50}$ titer was reported as GMT in reciprocal serum dilution. The results below the detection limit (dilution 1:20) were given a value of 10. According to the infection history and anti-N Ig, serum samples were categorized into five groups as follows: no infection (confirmed through seronegativity of anti-N Ig, n = 10), previous infection with the pre-Omicron dominant strain (infection history reported before January 2022, n = 8), the BA.1 Omicron dominant strain (infection history from January to March 2022, n = 12), the BA.5 Omicron dominant strain (infection history from July to September 2022, n = 12), and twice infection (across all period, n = 8). The timeframe of predominant strains in this study was assumed based on Thailand's COVID-19 endemic data, sourced from the GISAID website [21].

Statistical analysis

Baseline characteristics were presented as the mean with standard deviation (SD) or the median with interquartile range (IQR). Categorical age, sex, interval since the last dose, interval since the last dose or infection, and the follow-up day analyses were performed using Pearson's Chi-square test ($\chi 2$). The anti-RBD IgG and the $FRNT_{50}$ were reported as GMT with a 95 % confidence interval (CI). The geometric mean ratio (GMR) of the anti-RBD IgG between baseline (pre-boost) and post-boost for overall participants within vaccine dose was calculated using independent T-test. The GMRs of the anti-RBD IgG at post-boost between hybrid immunity and vaccine alone between the 3- to 6-doses group was analyzed by analysis of covariance (ANCOVA) with Bonferroni adjustment, with this test adjusting for the baseline titer. Differences of neutralizing antibody (nAb) between groups in were assessed using analysis of variance (one-way ANOVA). All statistical analysis was computed by IBM SPSS version 28 (IBM Corp., Armonk, NY), and a p-value < 0.05 was considered statistically significant.

Results

Demographic characteristics

A total of 123 healthy individuals were recruited to receive the booster dose of the BA.4/5 bivalent vaccine between July and August 2023. One individual was lost to follow-up. Raw data, including demographic characteristics, infection history, vaccination records, visit dates, laboratory assessments, and participant categorization, were provided in Supplementary Table S1. The individuals were initially classified according to the number of vaccine doses received before the BA.4/5 bivalent booster vaccination into five groups, ranging from 2- to 6-doses (hereafter referred to as the 2- to 6-doses groups). Within each group, individuals were further categorized based on their serostatus of anti-N Ig and infection history at pre-boost into a hybrid immunity group and a vaccine alone group. The vaccination records indicated that most participants (94.3 %) received administrations with various mix-and-match regimens of the ancestral Wuhan-H1 monovalent vaccines, which were developed by the Sinovac, Sinopharm, AstraZeneca, Pfizer–BioNTech, and Moderna companies. Only 5.7 % received homologous vaccine regimens. In parallel, the individuals were selected based on their history of infection and anti-N Ig serostatus to evaluate their neutralizing activity as shown in Fig. 1.

The demographic characteristics of the 2- to 6-dose groups including sex, age, underlying disease, the interval since the last dose, the interval since the last dose or infection, and the follow-up are described in Table 1. The percentage of females in the groups ranged from 50.0 % to 75.5 %, while the mean age ranged from 28.0 to 48.4 years across the groups. Most individuals underwent blood collection at 28 days post-booster. No significant differences were observed in terms of sex, age, and days of follow-up (p-value ≥ 0.05). The interval since the last dose was significantly longer in those who received fewer vaccines before the booster, with medians of 614.0, 520.5, 425.0, 361.0, and 209.0 days for the 2-to 6 −doses groups, respectively (p-value < 0.001).

The relationship between infection history and the serostatus of anti-N Ig

Out of the 122 participants, 54 (44.3 %) had no infection history, while 68 (55.7 %) had an infection history since the COVID-19 outbreak. Among the 68 cases with an infection history, 8 were assumed to have been infected with the pre-Omicron dominant strain (before January 2022), 8 participants reported being infected twice since the beginning of the COVID-19 outbreak, and the remaining 52 were assumed to had infections with the Omicron dominant strain (after January 2022), including two cases of negative anti-N Ig (Table 2 and Supplementary Table S1).

All individuals underwent testing for anti-N Ig and were classified into positive (N_{pos}) and negative (N_{neg}) results, with 90 (73.8 %) and 32 (26.2 %) cases, respectively. Subsequently, they were compared with their respective infection history. The findings indicated that 66 (54.1 %) and 30 (24.6 %) cases were identified as true positive and true negative of anti-N Ig in detecting past infections, respectively. However, 24 (19.7 %) cases were false positives for anti-N Ig, presumed to be asymptomatic infection, and 2 (1.6 %) cases were false negatives for anti-N Ig in detecting past infection at 462 and 485 days (Table 2 and Supplementary Table S1).

Post-boosted vaccination adverse events

Profiles of local, systemic and any adverse events within 6 days after vaccination with 50 μg mRNA-1273.222 were shown in Fig. 2. The most frequently solicited local events were injection site pain (88.5 %, 108/122), followed by swelling (23.8 %, 29/122). The highest incidence of systemic and any adverse events was myalgia (53.3 %, 65/122), followed by headache (33.6 %, 41/122) and chills (21.3 %, 26/122), respectively. After receiving a booster dose, overall adverse events were reported as mild to moderate and well-tolerated within a few days. A small number of individuals reported severe symptoms, ranging from 0.8 to 3.3 % (1 to 4 cases). There were no reports of vomiting, and there were no serious adverse events resulting in hospitalization or death.

No difference in antibody response elicited by BA.4/5 bivalent vaccine

In this study, the anti-RBD IgG levels were assessed following the BA.4/5 bivalent booster, comparing overall participants who had received 2- to 6-doses before obtaining the booster. The findings indicated comparable preexisting anti-RBD IgG with the GMTs ranging from

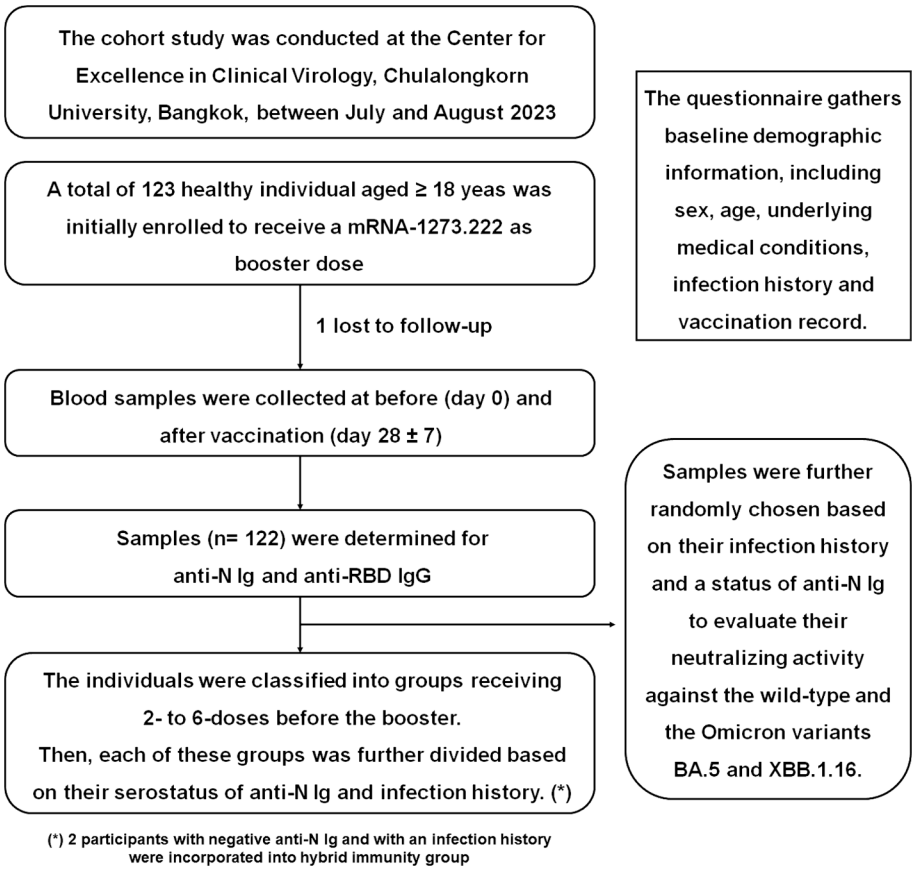

Fig. 1. Study flow diagram of the individuals who received a booster dose of 50-μg mRNA-1273.222.

Table 1
Baseline demographics and characteristics of enrolled participants.

	2 doses	3 doses	4 doses	5 doses	6 doses	*P*-value [¶]
Number (n)	3	24	41	39	15	
Female n (%)	2 (66.7)	12 (50.0)	31 (75.6)	27 (69.2)	10 (66.6)	0.223
Mean age [SD]	28.0 [16.5]	40.0 [17.3]	43.0 [13.1]	48.4 [15.3]	44.5 [14.1]	0.442
No comorbidity (%)	3 (100.0)	18 (75.0)	26 (63.4)	15 (41.0)	9 (7.5)	N/D
Underlying diseases (%)						
Allergy	—	4 (16.7)	5 (12.2)	6 (15.4)	—	N/D
Diabetes	—	—	2 (4.9)	3 (7.7)	1 (6.7)	N/D
Dyslipidemia	—	—	2 (4.9)	4 (10.2)	1 (6.7)	N/D
Hypertension	—	2 (8.3)	6 (14.6)	8 (20.5)	1 (6.7)	N/D
Other[#]	—	—	2 (4.9)	6 (15.4)	5 (3.3)	N/D
Interval since the last dose, days	614.0	520.5	425.0	361.0	209.0	<0.001
[IQR]	[585.0—617.0]	[377.5—539.0]	[356.0—534.0]	[291.0—399.0]	[209.0—209.0]	
Interval since the last dose or infection, days	614.0	390.0	365.0	340.0	209.0	0.003
[IQR]	[585.0—617.0]	[358.5—488.8]	[312.5—447.5]	[233.0—389.0]	[209.0—209.0]	
Follow-up, days	28.0	28.0	28.0	28.0	28.0	0.513
[IQR]	[28.0—28.0]	[28.0—28.8]	[28.0—29.0]	[28.0—28.0]	[28.0—28.0]	

[¶] The statistical analysis was conducted using Pearson's Chi-square test ($\chi 2$), and a p-value < 0.05 was considered statistically significant.
[#] Inactive diseases that did not require immunosuppressant medication were evaluated by a physician during the enrollment process. N/D, no determine. SD, standard deviation. IQR, Interquartile range.

725.9 to 1270 BAU/mL among the groups (Fig. 3A and Supplementary Table S2). After 28 days post-booster, the 3- to 6-dose groups showed a significant increase in anti-RBD IgG levels, with GMRs ranging from 4.7 to 7.3 (p-value < 0.001). In contrast, the 2-dose group showed no significant increase in IgG levels, with a GMR of 5.0, possibly due to the small sample size (n = 3).

In Fig. 3B, each group was categorized based on the serostatus of anti-N Ig at baseline into those with seronegative (defined as vaccine alone) and seropositive (defined as hybrid immunity). However, the 2 participants with seronegative anti-N Ig but with a history of test-confirmed infection were incorporated into the hybrid immunity group. At baseline, the results demonstrated that the hybrid immunity group remained at a higher level of preexisting immunity compared to the vaccine-alone group among the 3- to 5-dose groups, with the GMRs of 4.0, 4.8, and 7.4, respectively (all p-values ≤ 0.003). However, in the 6-dose group, a trend toward higher baseline anti-RBD IgG was observed

Table 2
The relationship between an infection history and the presence of total anti-N Ig.

Serostatus classified by total anti-N Ig		Infection history[#]		
		Yes	No	Total
	N_{pos} [¶]	66 (54.1 %)	24 (19.7 %)[*]	90 (73.8 %)
	N_{neg} [¶]	2 (1.6 %)[**]	30 (24.6 %)	32 (26.2 %)
	Total	68 (55.7 %)	54 (44.3 %)	122 (100.0 %)

[¶] The serostatus for total anti-N Ig was determined as positive (N_{pos}) when the results were equal to or greater than 1.0 COI, while results below 1.0 COI were considered negative (N_{neg}).
[#] The history of infection has been confirmed by RT-PCT and/or ATK.
[*] Individuals with the last exposure to inactivated vaccines (Sinovac) equal to or more than 748 days ago were assumed to be asymptomatic infections.
[**] These two participants have a confirmed history of a single infection; however, the serostatus is still seronegative, indicating a false negative anti-N Ig scenario.

with a GMR of 2.0 (no significance). At 28 days post-booster, the ratio of anti-RBD IgG comparing hybrid immunity to vaccine alone among the 3- to 6-dose groups ranged from 1.2 to 2.0 (Fig. 3B and Supplementary Table S2).

The BA.4/5 bivalent booster induced neutralizing antibodies corresponding to the vaccine strain and provided robust neutralization against the XBB.1.16 strain

To evaluate the neutralizing activity against the WT, BA.5, and XBB.1.16 strains in participants with no infection and those with a history of infection assumed to be with pre-Omicron, Omicron BA.1 and BA.5, and twice-infection, after receiving the BA.4/5 bivalent booster (Fig. 4). A subgroup of participants was randomly selected based on their infection history and status of anti-N Ig. All GMTs of nAb at pre- and post-boost with the BA.4/5 bivalent vaccine were provided in Supplementary Table S3.

At baseline, the interval since the last vaccination and infection was comparable among all subgroups, including no infection, pre-Omicron, BA.1, BA.5, and twice infection, with medians of 324.0, 408.5, 482.5, 327.0, 230.0 days, respectively (ns). Furthermore, the results confirmed that baseline nAb levels against WT, BA.5, and XBB.1.16 were similar across four hybrid immunity groups. The nAb titers against WT among individuals who had never been infected were lower than those who had hybrid immunity, in concordance with the anti-RBD IgG levels (Fig. 4A and Fig. 3B). Moreover, the nAb titers against the BA.5 and XBB.1.16 strains in the non-infected group were 20.1 and 9.6 in reciprocal serum dilution, respectively (Fig. 4B, C).

At 28 days post-boost within each group, the results demonstrated that the BA.4/5 bivalent vaccine robustly elicited nAb specific to the WT (GMRs ranging from 4.0 to 9.4, p-value < 0.05) and BA.5 (GMRs ranging from 3.6 to 21.7, p-value < 0.05), corresponding to the strains in the BA.4/5 bivalent vaccine (Fig. 4A-B). Interestingly, neutralizing activity against XBB.1.16 at 28 days was also observed (Fig. 4C). The findings indicated that the nAb titers were highest against WT, followed by BA.5 and XBB.1.16 strains, respectively.

To compare the nAb against WT across all five groups at post-boost, the results revealed no significant differences in GMR of nAb levels (Fig. 4A). The GMR of nAb against BA.5 after the booster also showed no significant difference among the groups, whereas individuals assumed to be infected with BA.5 exhibited more potent inhibition than non-infected individuals (GMR of 6.7, p-value of 0.005) (Fig. 4B). Moreover, we compared the nAb against the XBB.1.16, the study indicated that the GMR of nAb exhibited higher levels among individuals with hybrid immunity than those with vaccine-induced immunity. This was particularly evident in those assumed to be infected with the BA.5 strain and those who have been infected twice, compared to non-infected individuals, with GMRs of nAb were 10.7 (p-value of 0.002) and 8.9 (p-value of 0.023), respectively (Fig. 4C).

Discussion

The cohort study presented findings involving 122 participants with diverse infection histories and vaccination schedules. The finding indicated that the common incidences were injection site pain, myalgia, headache, swelling at the injection site, and chills, while other adverse events occurred less frequently. No severe adverse events leading to hospitalization or death among these participants were observed. This aligned with earlier studies that assessed the use of booster doses with the original monovalent mRNA vaccine [22–24] and a bivalent mRNA vaccine containing the beta variant [25]. These vaccines were developed using a similar strategy and the same components, but they differ in the mRNA sequence encoding the spike protein.

This study showed that documented infection history alone might not be sufficient to predict past infection because many individuals had asymptomatic infections. Combining anti-N Ig testing with infection history and other relevant data might be a useful marker for assessing hybrid immunity. The results revealed that 75.4 % of individuals

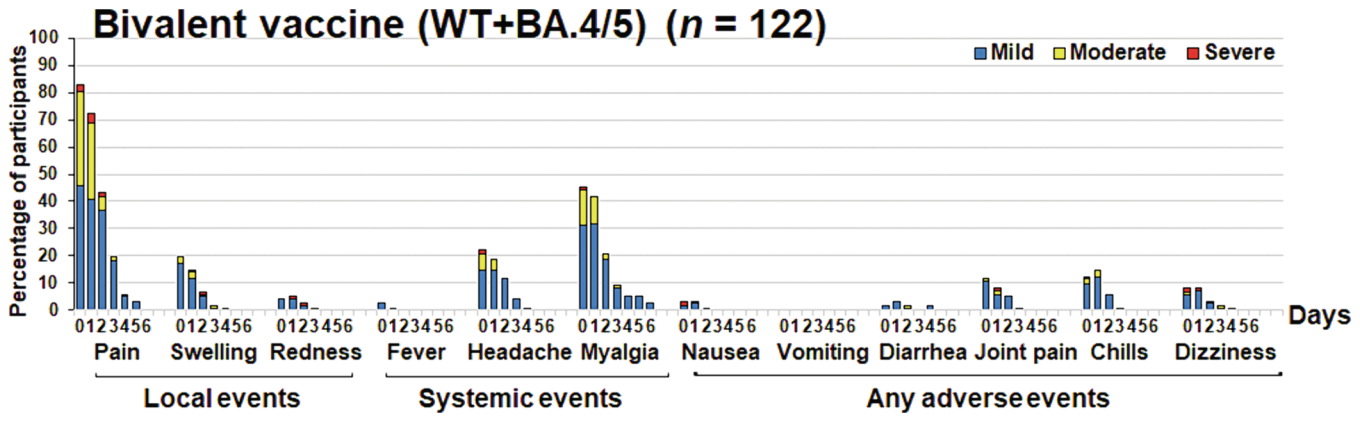

Fig. 2. The overall reactogenicity of individuals who received a booster dose of the bivalent mRNA-1273.222 was assessed, illustrating the percentages of local, systemic, and any adverse events that occurred within 6 days following the booster dose. Each adverse event was graded as mild, moderate, or severe.

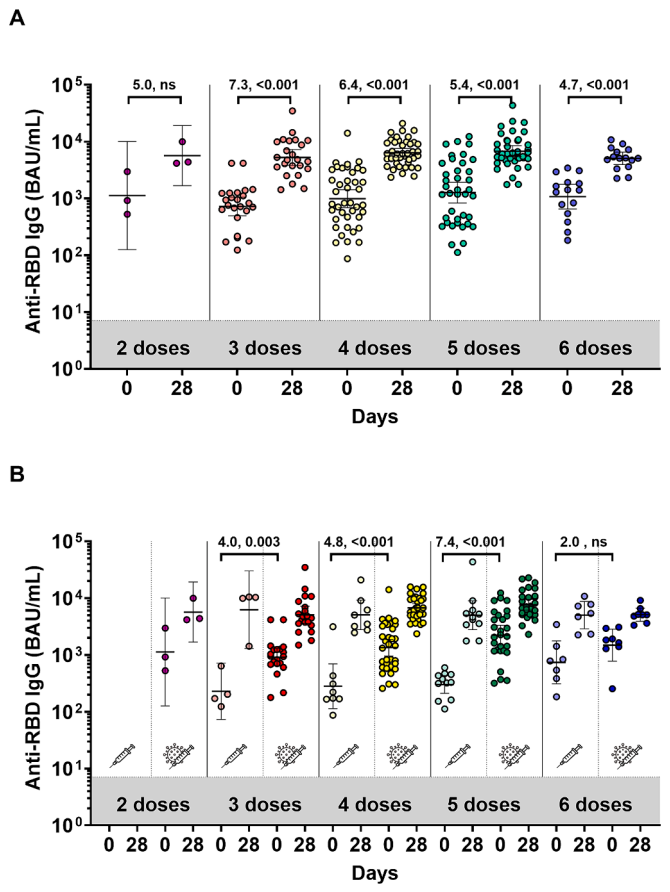

Fig. 3. The binding antibody against the SARS-CoV-2 RBD in individuals who received a booster with the BA.4/5 bivalent vaccine. A total of 122 enrolled individuals were divided into groups based on the number of the original COVID-19 vaccine they received (2, 3, 4, 5, and 6 doses, respectively) before the booster with the BA.4/5 bivalent Moderna vaccine. Anti-RBD IgG of overall individuals (A) and each group that is categorized based on serostatus of anti-N Ig consisting of negative and positive (B). Serum samples were collected for antibody testing at baseline (0) and 28 days post-vaccination (28). Lines represent the geometric mean titer (GMT) with 95 % confidence intervals (95 % CI). A pairwise comparison displays the geometric mean ratio (GMR) and significant value. The gray area indicates the seronegativity of anti-RBD IgG (<7.1 BAU/mL). A syringe logo indicates individuals with a negative result for anti-N Ig (vaccine alone), while a syringe with a virus logo indicates individuals with a positive result for anti-N Ig (hybrid immunity). The statistical difference was reported as a *p*-value < 0.05 and no significant difference (ns).

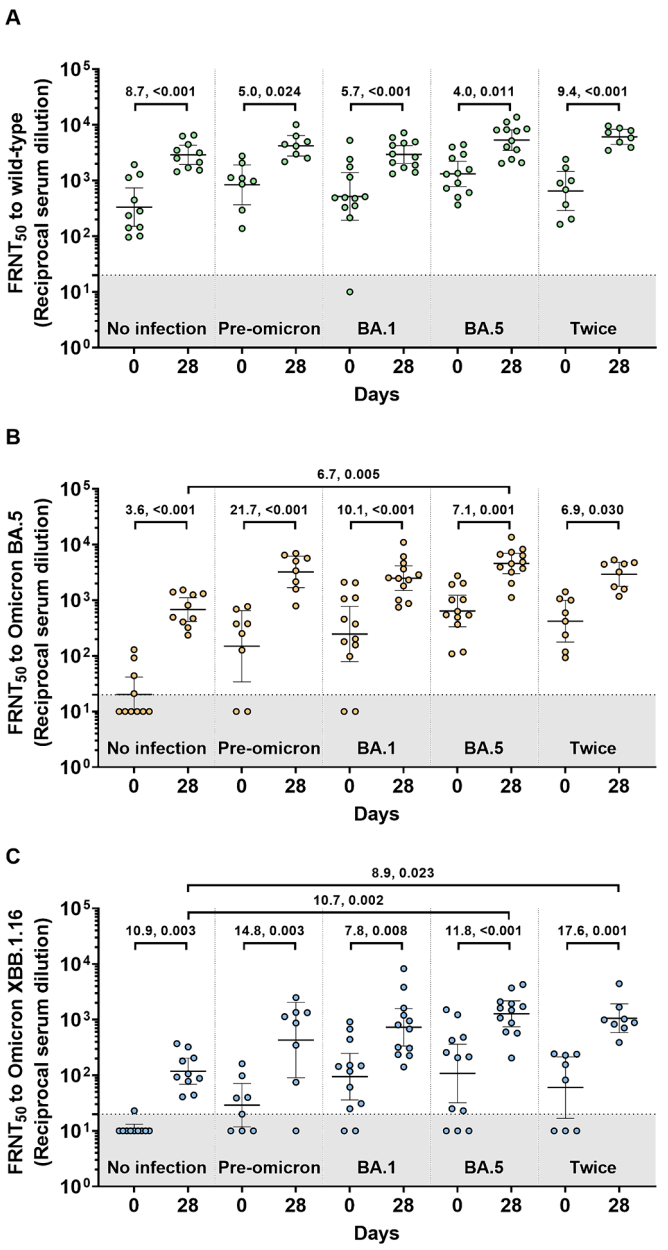

Fig. 4. The neutralizing activities among the individuals were classified into five groups based on infection history. The sera sample at baseline (0) and 28 days post the booster dose with the bivalent vaccine underwent the foci reduction neutralization test ($FRNT_{50}$) against the wild type (A) and Omicron BA.5 (B) and XBB.1.16 (C). Lines represent the geometric mean titer (GMT) with 95 % confidence intervals (95 % CI). A pairwise comparison displays a geometric ratio and a significant value. The gray area indicates the detection limit, with the dilutions below 20 considered negative. The statistical difference was reported as a *p*-value < 0.05.

exhibited hybrid immunity, as evidenced by the presence of anti-N Ig and infection history. This coincided with a previous serosurvey conducted in Chonburi province between October 2022 and January 2023 (73.7 %) and another study in Bangkok between April and June 2023 (71.7 %) [26,27]. This suggests that the cumulative infection rate of the Thai population is expected to increase slightly, given that a considerable number of individuals possess hybrid immunity.

In this cohort, we observed that that individuals with hybrid immunity had higher pre-boost levels of antibody compared to infection-naive individuals, which had been shown in other studies [27,28]. No detectable neutralizing activities against Omicron BA.5 and XBB.1.16 were observed in individuals without prior infection. Following the administration of the BA.4/5 bivalent vaccine booster, there was a significant increase in neutralizing antibody responses to the vaccine-related strain, including the WT and the Omicron BA.5, across all observed groups. Additionally, the study showed that the neutralizing antibody titer specific to the WT was higher than that for the BA.5. Our findings suggested that immune imprinting by previous antigenic exposure, particularly through receiving multiple doses of ancestral vaccines, leads to a more robust immune response against the WT compared to the BA.5. Our study aligned with the previous study, indicating that the BA.4/5 bivalent vaccine demonstrated a stronger neutralizing antibody response against the ancestral strain (with an increase from 3633 to 40,515) compared to the BA.5 variant (which increased from 212 to 3693) in persons with hybrid immunity (receiving 2 to 4 doses of ancestral vaccine) documented during the high prevalence of the BA.5 subvariant [29]. Similar observations were made in another study, indicating that the BA.4/5 bivalent vaccine more effectively elicited the neutralizing antibody response against the WT

compared to the Omicron subvariants in those who had a history of BA.4–BA.5 breakthrough infection after three or four doses of original monovalent mRNA vaccine [30]. Furthermore, our study observed neutralizing activity against the XBB.1.16 variant. This finding aligned with a related study, indicating that 83 % of a healthy cohort who received the BA.4/5 booster dose had detectable a neutralizing activity against the XBB.1.16 and XBB.1.9 subvariants [15]. In agreement with another study, it was demonstrated that the BA.4/5 bivalent booster elicited higher neutralizing activity against BF.7, BA.2.75.2, BQ.1.1, and XBB.1 subvariants in individuals with hybrid immunity than those without infection [31]. Taken together, this suggests that the BA.4/5 bivalent vaccine provides a good neutralizing antibody to the newly emerged Omicron sublinages.

On December 1st, 2023, the recommendations from the World Health Organization for COVID-19 vaccination state that there is not enough evidence to support getting vaccinated annually [32]. However, countries with established patterns of seasonality for other respiratory infections, like influenza, might consider getting vaccinated before the colder season. It's important to note that most healthy children, adolescents, and adults who have already been vaccinated and/or have had past COVID-19 infections are not currently recommended for revaccination. Adults aged 50 or 60 years and older with comorbidities may consider revaccination 6 to 12 months after the most recent dose. Moreover, it is recommended that individuals who have never received a COVID-19 vaccine, including healthy individuals, children, adolescents with underlying health conditions, and pregnant individuals, should get at least one dose. Immunocompromised individuals may require 2–3 doses after consulting with medical professionals [32]. It is widely acknowledged that vaccination can decrease the severity of COVID-19 and lower the experience of long-term post-acute symptoms (long COVID), with reductions ranging between 15 % and 40 % [33–35].

This study was subject to certain limitations. Viral sequencing data is unavailable to confirm the variants among these participants; however, we classified and selected the samples based on a comparison between the date of COVID-19 onset and the predominantly circulating variant reported at the time. Therefore, some cases in the studies pose a challenge in differentiating between subvariants. Some cases with asymptomatic infections were unaware of their infection history. This study suggests that additional studies may be conducted to enhance our understanding of vaccine effectiveness against infection and the severity of the disease. Moreover, long-term immune responses should be further focused on.

Conclusion

Our cohort study on the safety and antibody response of the Omicron BA.4/5 bivalent booster vaccine, administered to individuals with diverse vaccination histories, yielded several key findings. The booster dose was well-tolerated, with common adverse events being mild and limited to local and systemic reactions. Between July and August 2023, the prevalence of hybrid immunity, characterized by the presence of anti-N Ig, was notable among participants, reaching 73.8 %. This indicates higher baseline antibody levels compared to those with vaccination alone. The administration of the BA.4/5 bivalent vaccine as a booster elicited a significant increase in anti-RBD IgG response, particularly in neutralizing antibody responses against the WT and Omicron BA.5. Additionally, it exhibited neutralizing activity against the Omicron XBB.1.16 variant. Our findings contribute valuable insights to the ongoing efforts to manage COVID-19 vaccination strategies, particularly for individuals with diverse vaccination histories.

Institutional review board statement

The study protocol was approved by the Institutional Review Board (IRB), Faculty of Medicine, Chulalongkorn University (IRB number 284/66).

Informed consent statement

As all the data collected for final analysis in this study have been anonymized, the institutional review board of the Faculty of Medicine, Chulalongkorn agreed to its being made available on reasonable request to the corresponding author.

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CRediT authorship contribution statement

Sitthichai Kanokudom: Writing – review & editing, Writing – original draft, Project administration, Methodology, Formal analysis, Data curation. **Jira Chansaenroj:** Methodology, Formal analysis. **Nungruthai Suntronwong:** Formal analysis. **Lakkhana Wongsrisang:** Methodology. **Ratchadawan Aeemjinda:** Methodology. **Preeyaporn Vichaiwattana:** Methodology. **Thaksaporn Thatsanathorn:** Data curation. **Warangkana Chantima:** Methodology. **Pattarakul Pakchotanon:** Methodology. **Thaneeya Duangchinda:** Methodology. **Natthinee Sudhinaraset:** Data curation. **Sittisak Honsawek:** Writing – review & editing, Writing – original draft, Project administration. **Yong Poovorawan:** Writing – review & editing, Writing – original draft, Project administration, Conceptualization.

Declaration of competing interest

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.

Data availability

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jvacx.2024.100538.

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