



Heterologous Prime-boost of SARS-CoV-2 inactivated vaccine and mRNA BNT162b2 among Healthy Thai Adolescents



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ABSTRACT

Background: Heterologous prime-boost SARS-CoV-2 vaccination is a widely accepted strategy during the COVID-19 pandemic, which generated a superior immune response than homologous vaccination strategy.

Objective: To describe immunogenicity of heterologous prime-boost vaccination with inactivated vaccine, CoronaVac, followed by BNT162b2 and 5-month booster dose with BNT162b2 in healthy Thai adolescents.

Methods: Adolescents aged 12–18 years were randomized 1:1:1 to receive CoronaVac (SV) followed by BNT162b2 (PZ) 30 or 20 µg at either 3- or 6-week interval (SV3w/PZ30µg, SV3w/PZ20µg, SV6w/PZ30µg or SV6w/PZ20µg). During the Omicron-predominant period, participants were offered a BNT162b2 booster dose 30, 15, or 10 µg. Immunogenicity was determined using IgG antibody against spike-receptor-binding domain of wild type (anti-S-RBD IgG) and surrogate virus neutralization test (sVNT) against Delta variant at 14 days and 5 months after the 2nd dose. Neutralization tests (sVNT and pseudovirus neutralization test; pVNT) against Omicron strain were tested pre- and 14 days post-booster dose.

Results: In October 2021, 76 adolescents with a median age of 14.3 years (IQR 12.7–16.0) were enrolled: 20 in SV3w/PZ30µg; 17 in SV3w/PZ20µg; 20 in SV6w/PZ30µg; 19 in SV6w/PZ20µg. At day 14, the geometric mean (GM) of anti-S-RBD IgG in SV3w/PZ30µg was 4713 (95 %CI 4127–5382) binding-antibody unit (BAU)/ml, while geometric mean ratio (GMR) was 1.28 (1.09–1.51) in SV6w/PZ30µg. The GMs of sVNT against Delta variants at day 14 among participants in SV3w/PZ30µg and SV6w/PZ30µg arm were 95.3 % and 99.7 % inhibition, respectively. At 5 months, GMs of sVNT against Delta variants in SV3w/PZ30µg were significantly declined to 47.8 % but remained at 89.0 % inhibition among SV6w/PZ30µg arm. In April 2022, 52 adolescents received a BNT162b2 booster dose. Proportion of participants with sVNT against Omicron strain > 80 % inhibition was significantly increased from 3.8 % pre-booster to 67 % post-booster. Proportion of participants with pVNT ID₅₀ > 185 was 42 % at 14 days post 2nd dose and 88 % post booster, respectively.

Abbreviations: BAU, Binding-antibody unit; CMI, Cell-mediated immune response; ELISpot, Enzyme-linked immunospot; GM, Geometric mean; GMR, Geometric mean ratio; ID₅₀, Neutralization dilution for 50% pseudovirus inhibition; PBMC, Peripheral blood mononuclear cell; pVNT, Pseudovirus neutralization test; SFU, Spot forming unit; S-RBD, Spike receptor binding domain; sVNT, Surrogate virus neutralization test.

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Conclusions: Heterologous prime-boost vaccination with CoronaVac followed by BNT162b2 induced high neutralizing titer against SARS-CoV-2 Delta strain. After 5-month interval, booster with BNT162b2 induced high neutralizing titer against Omicron strain.

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Introduction

The inactivated vaccine, CoronaVac (Sinovac Life Sciences, Beijing, China), was derived from whole virus of SARS-CoV-2 [1], while BNT162b2 (Pfizer–BioNTech) is an mRNA encoding the SARS-CoV-2 full-length spike protein vaccine [2]. The heterologous prime-boost vaccination with CoronaVac and BNT162b2 as primary series has been used in adults in Thailand, which demonstrated comparable anti-receptor-binding-domain (anti-RBD) IgG and surrogate virus neutralization test (sVNT) against wild type and Delta variant at 4 weeks after 2nd dose to homologous BNT162b2 regimen [3]. Also, the heterologous booster in 2-dose CoronaVac vaccinees using AZD1222 (ChAdOx1 nCoV-19, AstraZeneca) or BNT162b2 has been used in adult program in Thailand [4,5]. Heterologous prime boost vaccination strategy could benefit in terms of increased coverage during vaccine shortages and might lessen the concern of myocarditis after COVID-19 mRNA vaccinations in children.

CoronaVac was demonstrated to be safe, able to induce humoral immune responses [6], and effective against severe COVID-19 in children and adolescents [7]. CoronaVac has been approved for emergency use by World Health Organization (WHO) on June 1, 2021 [8] and approved for emergency use in children and adolescents in several countries including Thailand [9], China [10] and Latin Americas with >8 million doses administered in March 2021 [11]. The vaccine effectiveness of CoronaVac in children in Chile was 75% for COVID-19 infection and over 90% for hospitalization, during the period of Delta predominance [7].

In children and adolescents, the fractional low-dose BNT162b2 could be as immunogenic as full dose in adults. A dosage of 10- μ g BNT162b2 has been recommended for use in children aged between 5 and 11 years by the U.S. Advisory Committee on Immunization Practices [12]. In adolescents, 2 doses of 30- μ g BNT162b2 were able to induce higher neutralizing antibody titers in adolescents aged 12–15 years than young adults aged 16–25 years, with geometric mean ratio (GMR) of 1.76 (95% CI 1.47–2.10) [2]. Thus, a dosage of 20 μ g might be reasonable for adolescents with robust immune responses.

Since SARS-CoV-2 Omicron variant (B.1.1.529)-predominant COVID-19 pandemic in January 2022 [13], booster vaccinations are needed. The Omicron variant contains 15 amino acid changes in the receptor binding domain (RBD) [14], resulting in lower neutralizing activities [15] and lower vaccine effectiveness [16]. A BNT162b2 booster dose was authorized by the United States Food and Drug Administration for booster in healthy adolescents with an interval of at least 5 months after the primary series [17], and recommended by The Advisory Committee on Immunization Practices (ACIP) [18]. CoronaVac is produced from whole virus of SARS-CoV-2 [1], while BNT162b2 is an mRNA vaccine encoding the SARS-CoV-2 spike protein [2]. Heterologous prime-boost vaccination with these two different vaccine platforms might induce broader immune responses to SARS-CoV-2 and SARS-CoV-2 variants.

Therefore, this study aimed to describe the immunogenicity of heterologous prime-boost vaccination with inactivated vaccine CoronaVac and low-dose BNT162b2, and additional 5-month boosters against Omicron variant among healthy adolescents in Thailand.

Method

Study design and participants

This is a double-blinded, randomized, phase 2 clinical trial, conducted at Center of Excellence for Pediatric Infectious Diseases and Vaccines, Chulalongkorn University, Bangkok, Thailand, in October 2021. Healthy participants aged 12 to < 18 years without previous SARS-CoV-2 vaccination were recruited and screened for eligibility. The exclusion criteria were participants with known anaphylaxis to any of the vaccine components or drugs, previous SARS-CoV-2 infection by history and/or positive anti-nucleocapsid IgG, receipt of immunoglobulins or blood products within 3 months of the first vaccination, receipt of any other vaccines within 14 days (for inactivated vaccines) or 28 days (for live vaccines) before enrollment, or who were immunosuppressed. Informed consent was given and signed by parent or legally authorized representative, while participants signed a written informed assent form. Primary vaccination series with CoronaVac and BNT162b2 30 μ g or 20 μ g were given to all participants. In March 2022, with a wide spread of Omicron variant in Thailand, we amended the protocol to add sub study of a booster dose, BNT162b2 30 μ g, 15 μ g, or 10 μ g, by double-blinded randomization. The institutional review board of Faculty of Medicine, Chulalongkorn University approved this study (IRB no. 827/64). This study was registered in thaiclinicaltrials.org (TCTR20210923012).

Study procedure

A total of 80 participants were recruited. After assessing for inclusion and exclusion criteria, 5 ml of blood were collected prior to the first vaccination at the first visit, to test for anti-nucleocapsid IgG, to exclude participants confirmed to have had recent natural infection. Participants were randomized to be vaccinated with CoronaVac (SV) 3 μ g (equivalent to 600 SU per 0.5 ml), lot number C202105098, as the first dose followed by BNT162b2 30 μ g (0.3 ml, PZ30) or 20 μ g (0.2 ml, PZ20), lot number 30125BA, as the second dose, at 3-week (3w) or 6-week interval (6w). In total, there are 4 vaccination groups: 3-week-interval CoronaVac/BNT162b2 30 μ g (SV3w/PZ30) as reference and other 3 comparing regimens, 3-week-interval CoronaVac/BNT162b2 20 μ g (SV3w/PZ20), 6-week-interval CoronaVac/BNT162b2 30 μ g (SV6w/PZ30), and 6-week-interval CoronaVac/BNT162b2 20 μ g (SV6w/PZ20). The visits were scheduled as follows: day 1 for the first dose vaccination; day 21 or 42 for the second dose vaccination; 14 days, 60 days, and 5 months after complete vaccination to perform blood collection for immunogenicity testing and collect history of COVID-19 infection and unsolicited adverse events. Laboratory assays on immunogenicity against Delta variant and wild type were performed at all visits after vaccination by surrogate virus neutralization test (sVNT) and anti-spike-receptor-binding domain of wild type (anti-S-RBD IgG-wild type).

During 7 days post each vaccination, the solicited local and systemic reactogenicities [19] were recorded by participants using structured diary. The solicited local reactogenicities included pain at injection site, swelling, and erythema, and the solicited systemic reactogenicities included fever, vomiting, diarrhea, headache, fati-

gue, myalgia, and arthralgia. All adverse events were graded by severity into mild (grade 1), moderate (grade 2), severe (grade 3), and potentially life-threatening (grade 4), according to Guidance for Industry by the United States Food and Drug Administration [19]. Local swelling and redness were graded by size: 2.5–5 cm, mild (grade 1); 5.1–10 cm, moderate (grade 2); > 10 cm, severe (grade 3); necrosis, potentially life-threatening (grade 4). Fever was classified by the peak body temperature as mild (38.0–38.4 °C), moderate (38.5–38.9 °C), severe (39.0–40.0 °C), and potentially life-threatening (>40.0 °C). Vomiting for 1–2 episodes/day or diarrhea for 2–3 stools/day was categorized as mild, > 2 episodes/day or 4–5 stools/day as moderate, as severe if required outpatient intravenous hydration, and as potentially life-threatening if required hospitalization for hypotensive shock. The other reactogenicities were graded as mild if no interference with activity, moderate if some interference with activity, severe if precluding daily activity, and potentially life-threatening if hospitalization.

Cell-mediated immune responses (CMI) were assessed in 10 participants per group, or a total of 40 participants (CMI sub study). Additional 8-ml blood samples were collected at 14 days, 60 days, and 5 months after complete vaccination to test for T cell responses by enzyme-linked immunospot (ELISpot) assay. Humoral immune responses by B cell ELISpot assay were also tested in these participants at 5 months after complete vaccination.

In April 2022, all eligible participants were invited to participate in the booster sub study. Participants were randomized to receive a booster dose with BNT162b2 30 µg (0.3 ml, PZ30) or 15 µg (0.15 ml, PZ15), lot number PCA0062, or pediatric BNT162b2 10 µg (0.2 ml, PZ10), lot number FN4074, at 5 months post primary series visit after blood sample collection. Due to a wide spread of Omicron variant in Thailand at the time, the immunogenicity against Omicron variant was assessed in the booster sub study at 14 days post 2nd dose (the prior collected samples), 5 months post 2nd dose (pre booster) and 14 days post booster dose by sVNT, and at 14 days post 2nd dose and post booster dose by pseudovirus neutralization test (pVNT).

Immunogenicity outcomes

Immunogenicity was assessed using IgG antibody against spike-receptor-binding domain of wild type (anti-S-RBD IgG-wild type) and functional neutralizing antibody (NAb) against SARS-CoV-2 wild type, Delta, and Omicron variants by surrogate virus neutralization test (sVNT) and pseudovirus neutralization test (pVNT). The geometric mean ratio (GMR) of each arm was compared with the 3-week-interval 30-µg BNT162b2 arm (SV3w/PZ30) as the reference arm, the standard interval and dose arm.

Quantitative spike receptor binding domain IgG against wild type (anti-S-RBD IgG-wild type) ELISA

The ELISA protocol was modified from Amanat *et al.* [20] and performed as described previously [21,22]. Briefly, the ELISA plates were coated with purified recombinant Myc-His-tagged S-RBD, residues 319–541 from SARS-CoV-2 (Wuhan-Hu-1). Participants' sera were diluted 1:5000 in blocking buffer and used as primary antibodies. HRP-conjugated human IgG was used as a secondary antibody. After addition of TMB substrate, OD450 was measured for each sample and converted into binding-antibody units (BAU/mL) of anti-S-RBD IgG, using the standard curve of known units of WHO international standard (NIBSC 20/136).

Surrogate virus neutralization test (sVNT)

The surrogate virus neutralization test was adjusted from Tan *et al.* (2020) [23] and performed as described previously [21,22], utilizing the HRP-tagged recombinant SRBD from the wild-type (Wuhan-Hu-1), Delta (B.1.617.2), and Omicron (B.1.1.529 – BA.1)

strains. The 96-well plates coated with 0.1 µg/well purified recombinant human ACE2 ectodomain were used to incubate with serum samples (at 1:10 dilution) - SRBD mixture. Then, ELISA was performed by incubating the mixture with the hACE2 coated plates for one hour and adding TMB substrate with ample washing in between. OD450 was measured. Pre-2019 human serum was used as the negative sample. The % inhibition was calculated as follows:

$$\%inhibition = 100 \times \left[1 - \frac{sample\ OD450}{negative\ OD450} \right]$$

Pseudovirus neutralization test (pVNT)

Pseudovirus neutralization test (pVNT) against the Omicron variant was performed as described previously [24]. Twofold serial dilutions of serum samples (starting 1:40) were incubated with pseudoviruses displaying the Omicron (B.1.1.529; BA.2) spike in a 1:1 vol/vol ratio in a 96-well culture plate for 1 h at 37 °C. The pseudovirus input used was normalized to 1×10^5 RLU/well. Subsequently, suspensions of HEK293T-ACE-2 cells (2×10^4 cells/ml) were mixed with the serum-pseudovirus mixture and seeded into each well. At 48 h, the neutralizing antibodies were determined based on luciferase activity following entry of pseudovirus. Values were normalized against signals from no-serum controls. The ID₅₀ values were calculated by determining the half-maximal inhibitory dilution. We used pVNT ID₅₀ at 185, which correlated with 80 % vaccine efficacy of ChAdOx1 nCoV-19 vaccine (Oxford/AstraZeneca) [25], as a cut off.

Enzyme-linked immunospot (ELISpot) assay to evaluate T and B cell responses

For T cell, ELISpot assay by a Human IFN-γ ELISpotPro™ kit (Mabtech, Stockholm, Sweden) was used for assessing T cell responses. Freshly isolated peripheral blood mononuclear cells (PBMCs) with 250,000 cells per well were activated with 2 µg/ml of overlapping peptide pool from 100 peptides of SARS-CoV-2 spike (S)-defined peptides (Mabtech, Stockholm, Sweden) for 20 h. Negative control and positive control, anti-CD3, were also included. The spots were quantified with ImmunoSpot analyzer. To evaluate positive S peptide-specific responses, spot counts of negative control wells were subtracted from S peptide-stimulated wells, and these spot counts are reported as spot forming unit (SFU) per million PBMCs.

For B cells, Human IgG SARS-CoV-2 RBD ELISpot PLUS (ALP) kit (Mabtech, Stockholm, Sweden) was used to assay for SARS-CoV-2-specific memory B cell responses. Briefly, the memory B cells were enriched by pre-stimulating the fresh PBMCs with R848 and IL-2 for 72 h. Unstimulated well was also used as negative control. Stimulated and unstimulated PBMCs (5×10^5 cells per well) were added into ELISpot plate coated with capture anti-IgG antibody and incubated for 18 h. Different labels were used for test and control, in which an RBD-WASP antigen was added into RBD-specific IgG detected well while MT78/145- biotinylated antibodies were added into positive control (total IgG detected well). Therefore, anti-WASP-ALP was added into RBD-specific IgG detected well and negative control well while streptavidin-ALP was added into positive control well. Spot counting was performed using the same method as T cells.

Statistical analysis

The sample size was calculated using a non-inferiority criterion for the geometric mean ratio (GMR) of sVNT against Delta variant at day 14 after complete primary vaccination series of SV3w/PZ20, SV6w/PZ30, and SV6w/PZ20, using SV3w/PZ30 as the reference arm. Assuming 0.67 non-inferiority margin, 80 % power, 0.20 geo-

metric standard deviation, 0.95 GMR, one-sided statistical testing with 5 % significant level, and ratio 1:1, a minimum of 17 participants per group was required. Accounting for potentially missing data, the sample size was increased by 20 %, yielding a total of 20 participants per group.

Demographic and clinical characteristics were described. Continuous variables were expressed as median (interquartile range, IQR) and number with percentage for categorical variables. Differences in continuous and categorical variables between two groups were assessed using a Wilcoxon rank sum test, Chi-square test, or Fisher exact test, respectively. The primary outcomes were GMRs of sVNT against Delta variant and anti-S-RBD IgG-wild type at 14 days after primary vaccination series of each group using SV3w/PZ30 as the reference arm. The exploratory outcomes included immunogenicity against Omicron variant by sVNT and pVNT after booster. Geometric means (GMs) and GMRs with 95 % CI of anti-S-RBD IgG, sVNT, and pVNT were calculated by two independent sample *t*-test. Non-inferiority was concluded if the lower bound of the 95% CI did not exceed 0.67. All P-values reported are two-sided. Statistical significance was defined as $P < 0.05$. Stata version 15.1 (Stata Corp., College Station, Texas) was used for analysis.

Results

Baseline characteristics

In October 2021, 80 healthy adolescents were enrolled and tested for anti-nucleocapsid IgG. Four participants (5%) had positive anti-nucleocapsid antibodies, therefore met exclusion criteria of prior COVID-19 infection. A total of 76 participants were

included and randomized to 4 groups: SV3w/PZ30, SV3w/PZ20, SV6w/PZ30, and SV6w/PZ20, as shown in Fig. 1. The mean age (SD) was 14.5 (1.9), 14.1 (1.6), 14.2 (1.4), and 15.1 (2.1) years, respectively. Males accounted for 50 %, 47 %, 85 %, and 68 % of the participants, respectively. During the 5-month follow-up period, 5 participants had symptomatic COVID-19 upper respiratory tract infection during the Omicron-dominant period, as shown in Supplementary Table 1. All of them occurred after 3 months from BNT162b2 dose. These participants were excluded from the immunogenicity analysis at the time points after acquiring COVID-19 for all immunogenicity outcomes and CMI sub study, and not eligible for participating in booster sub study.

At month 5, 52 participants consented to participate in the booster sub study, 7 participants planned to receive a booster dose from the national program, while 12 participants preferred not to get a booster dose.

Reactogenicity after CoronaVac (SV) and BNT162b2 (PZ) primary series and booster

After SV vaccination as the first dose, 42–65 % of participants experienced pain at injection site with mild-to-moderate severity, as shown in Fig. 2A. Also, only mild-to-moderate myalgia, fatigue, and headache were reported in 15–43 %, 20–41 %, and 10–35 % of participants, respectively. Post BNT162b2 vaccination as the second dose, 75–89 % of participants had local pain, graded as severe pain in 5–15 %, as shown in Fig. 2B. Mild-to-moderate myalgia, headache, fatigue, and fever were reported in 45–58 %, 20–55 %, 30–50 %, and 0–15 % of the participants, respectively.

After BNT162b2 booster vaccination, PZ10 recipients tended to have fewer reactogenicities and less severity, compared with PZ15

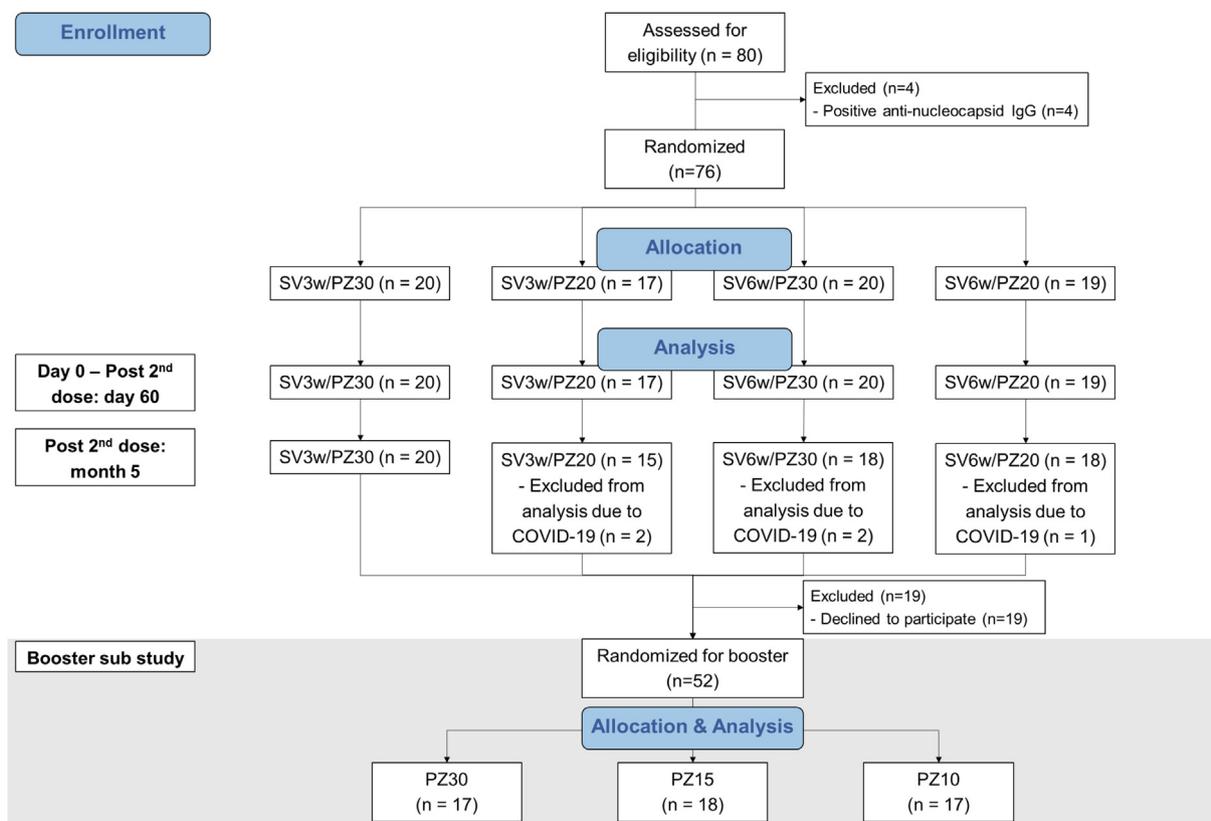


Fig. 1. Flow diagram of study participants. PZ10: Booster with BNT162b2 10 µg; PZ15: Booster with BNT162b2 15 µg; PZ30: Booster with BNT162b2 30 µg; SV3w/PZ30: 3-week-interval CoronaVac/BNT162b2 30 µg; SV3w/PZ20: 3-week-interval CoronaVac/BNT162b2 20 µg; SV6w/PZ30: 6-week-interval CoronaVac/BNT162b2 30 µg; SV6w/PZ20: 6-week-interval CoronaVac/BNT162b2 20 µg.

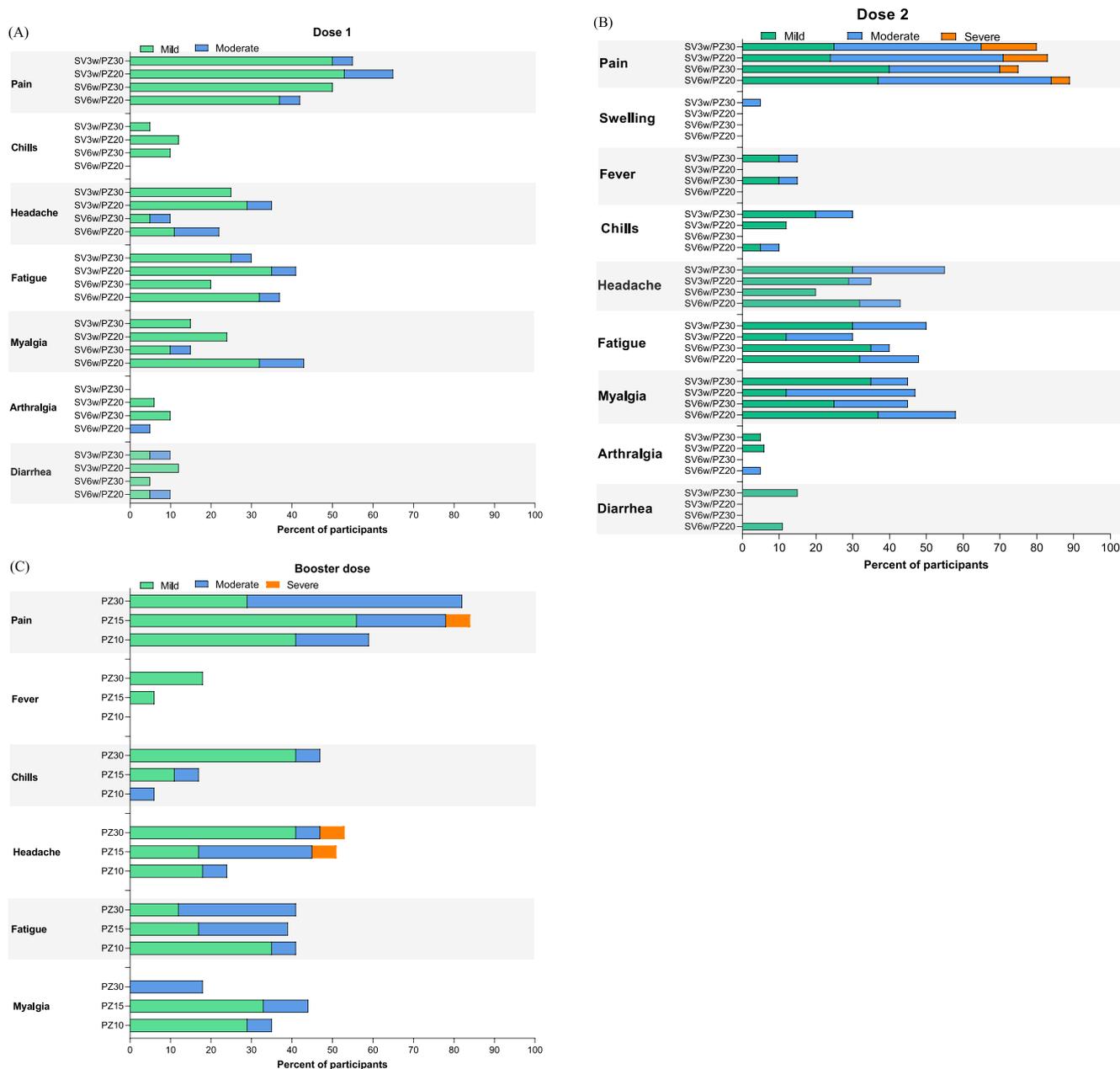


Fig. 2. Local and systemic reactogenicities post vaccination in healthy adolescents by vaccination group: (A) after CoronaVac vaccination (1st dose), (B) after BNT162b2 vaccination (2nd dose), and (C) after BNT162b2 booster vaccination. PZ10: Booster with BNT162b2 10 µg; PZ15: Booster with BNT162b2 15 µg; PZ30: Booster with BNT162b2 30 µg; SV3w/PZ30: 3-week-interval CoronaVac/BNT162b2 30 µg; SV3w/PZ20: 3-week-interval CoronaVac/BNT162b2 20 µg; SV6w/PZ30: 6-week-interval CoronaVac/BNT162b2 30 µg; SV6w/PZ20: 6-week-interval CoronaVac/BNT162b2 20 µg.

and PZ30 groups e.g., pain at injection site (59 % vs 82–84 %), and headache (24 % vs 50–53 %), as shown in Fig. 2C. No fever was reported in PZ10 recipients. Detailed information of reactogenicity after all vaccination doses were reported in Supplementary Table 2.

Immunogenicity after heterologous prime-boost vaccination with CoronaVac (SV) and BNT162b2 (PZ)

Anti-S-RBD IgG-wild type

The results of anti-S-RBD IgG-wild type are shown in Table 1 and Fig. 3A. After BNT162b2 vaccination as the 2nd dose, anti-S-RBD IgG-wild type was markedly boosted, with GM of 4713 BAU/ml (95 % CI 4127–5382) in SV3w/PZ30 at day 14, and GMR of 0.95 (95 % CI 0.80–1.13) in SV3w/PZ20, 1.28 (1.09–1.51) in SV6w/PZ30, and 1.10 (0.94–1.30) in SV6w/PZ20. The GMs of anti-

S-RBD IgG-wild type of 6-week interval arms were significantly higher than SV3w/PZ30 from 14 days after 2nd dose onwards in SV6w/PZ30 and 5 months onwards in SV6w/PZ20, with GMR of 1.50 (95 % CI 1.12–2.02) at day 60 and 2.37 (1.61–3.48) at month 5 in SV6w/PZ30, and 1.76 (1.19–2.61) at month 5 in SV6w/PZ20.

sVNT against Delta variant and wild type

The GMs of sVNT against Delta variant after vaccination are shown in Table 1 and Fig. 3B. The neutralizing antibody levels were markedly boosted after BNT162b2 vaccination for 14 days, with GM of 95.3 % inhibition (95 % CI 91.6–99.3) in SV3w/PZ30, and waned after 60 days and 5 months, with GMs of 74.2 % inhibition (67.1–82.0) and 47.8 % inhibition (38.6–59.3), respectively. Both 6-week interval groups had higher GMs of sVNT against Delta variant than SV3w/PZ30 from 60 days after 2nd dose vaccination, with

Table 1
Immunogenicity responses after CoronaVac/BNT162b2 vaccination as primary series in healthy adolescents by vaccination group.

Immunogenicity outcomes	SV3w/PZ30 (n = 20)	SV3w/PZ20 (n = 17)	SV6w/PZ30 (n = 20)	SV6w/PZ20 (n = 19)
Anti-S-RBD IgG-wild type (BAU/ml)				
- Post 1 st dose: 3–6 weeks, GM (95 % CI)	77 (56–106)	84 (71–101)	112 (82–154)	74 (51–107)
- Post 2 nd dose: day 14, GM (95 % CI)	4713 (4127–5382)	4489 (3980–5062)	6042 (5550–6577)	5200 (4483–6032)
- Post 2 nd dose: day 60, GM (95 % CI)	1116 (878–1419)	1182 (975–1435)	1674 (1380–2032)	1328 (1020–1728)
- Post 2 nd dose: month 5, GM (95 % CI)	415 (309–559)	613 (436–860)	982 (767–1259)	732 (535–1003)
sVNT against Delta variant (%inhibition)				
- Post 1 st dose: 3–6 weeks, GM (95 % CI)	12.8 (8.1–20.1)	6.6 (4.1–10.5)	24.9 (18.4–33.6)	17.5 (12.6–24.2)
- Post 2 nd dose: day 14, GM (95 % CI)	95.3 (91.6–99.3)	97.1 (95.9–98.2)	99.7 (95.4–100.0)	98.6 (96.8–100.4)
- Post 2 nd dose: day 60, GM (95 % CI)	74.2 (67.1–82.0)	76.5 (67.7–86.5)	96.0 (93.5–98.5)	85.7 (76.1–96.6)
- Post 2 nd dose: month 5, GM (95 % CI)	47.8 (38.6–59.3)	61.6 (50.6–75.1)	89.0 (84.4–93.7)	70.8 (59.0–85.1)
sVNT against wild type (%inhibition)				
- Post 1 st dose: 3–6 weeks, GM (95 % CI)	15.1 (7.6–29.9)	20.1 (14.6–27.6)	33.8 (24.8–46)	27.2 (21.3–34.8)
- Post 2 nd dose: day 14, GM (95 % CI)	98.3 (97.2–99.5)	99.0 (98.5–99.5)	99.9 (99.8–100.0)	99.6 (99.2–99.9)
- Post 2 nd dose: day 60, GM (95 % CI)	81.9 (75.5–88.8)	85.1 (79.3–91.4)	97.8 (95.9–99.7)	90.9 (84.4–97.9)
- Post 2 nd dose: month 5, GM (95 % CI)	58.9 (48.4–71.7)	70.5 (60.0–82.9)	95.0 (91.6–98.4)	77.4 (66.4–90.1)

Anti-S-RBD IgG: Anti-spike-receptor-binding-domain immunoglobulin G; GM: Geometric mean; SV3w/PZ30: 3-week-interval CoronaVac/BNT162b2 30 µg; SV3w/PZ20: 3-week-interval CoronaVac/BNT162b2 20 µg; SV6w/PZ30: 6-week-interval CoronaVac/BNT162b2 30 µg; SV6w/PZ20: 6-week-interval CoronaVac/BNT162b2 20 µg; sVNT: Surrogate virus neutralization test.

*p < 0.05.

GMR of 1.29 (95 % CI 1.14–1.47) in SV6w/PZ30 and 1.16 (1.02–1.32) in SV6w/PZ20 after 60 days, and GMR of 1.86 (1.49–2.33) in SV6w/PZ30 and 1.48 (1.18–1.86) in SV6w/PZ20 after 5 months. Low-dose BNT162b2 could induce non-inferior sVNT against Delta variant. The neutralizing antibody levels against wild type as measured by sVNT were greater than against Delta variant at all time points, as shown in Table 1.

T and B cell responses by ELISpot assay

Spike-specific T cell response and RBD-specific memory B cell response were demonstrated by ELISpot assay, as shown in Table 2. SV and BNT162b2 primary series could induce T cell responses, with median of 290–504 SFU/10⁶ PBMCs at day 14, 104–128 SFU/10⁶ PBMCs at day 60, and 56–120 SFU/10⁶ PBMCs at month 5. The median of memory B cell response was 8–25 SFU/10⁶ PBMCs at month 5. Both T and memory B cell responses were not different among vaccination groups (p > 0.05).

Immunogenicity against Omicron variant after BNT162b2 (PZ) booster

In April 2022, during a period of Omicron predominance, 52 participants received BNT162b2 booster dose with the median interval of 133 days (IQR 126–147) from previous dose of BNT162b2. The proportion of participants with neutralizing antibody against Omicron variant, measured by sVNT, over 80 % was 9.6 % at 14 days after primary series, declined to 3.8 % before booster and increased to 64.7 %, 72.2 %, and 64.7 % after booster with PZ30, PZ15, and PZ10, respectively, as shown in Fig. 4A. Pseudovirus neutralization assay against Omicron variant was done at 14 days after 2nd dose and after booster. Proportion of participants with pVNT against Omicron variant ID₅₀ > 185 was significantly increased from 42.3 % at day 14 after 2nd dose to 82.4–94.1 % in all booster arms (PZ30 = 94.1 %, PZ15 = 88.9 %, PZ10 = 82.4 %). The GMs of pVNT against Omicron variant ID₅₀ were comparable in all booster group, with GM of 552 (95 % CI 356–855) in PZ30,

and GMRs of 0.82 (0.44–1.53) in PZ15 and 0.74 (0.39–1.39) in PZ10, as shown in Fig. 4B.

Prior to BNT162b2 boosters, the GMs were 69.9 % inhibition (95 % CI 63.2–77.2) for sVNT against Delta variant, and 665 BAU/ml (566–782) for anti-S-RBD IgG-wild type, in participants of booster sub study. After booster with 30-µg BNT162b2, the GMs of sVNT against Delta variant were 99.7 % inhibition (99.6–99.8). Low-dose BNT162b2 booster, 15 and 10 µg, provided similar sVNT against Delta variants, with GMR of 1.00 (95 % CI 1.00–1.01) and 1.00 (95 % CI 0.99–1.00), respectively. The GM of anti-S-RBD IgG-wild type was 2828 BAU/ml (95 % CI 2545–3143) after standard-dose BNT162b2 booster for 14 days. The GMRs of anti-S-RBD IgG-wild type after 15- and 10-µg BNT162b2 booster were 0.91 (95 % CI 0.73–1.13) and 0.73 (0.58–0.91), respectively.

Discussion

Heterologous prime-boost vaccination with inactivated vaccine SV and mRNA vaccine BNT162b2 in adolescents was found to be safe and immunogenic, particularly favoring a 6-week interval regimen. Using 20-µg BNT162b2 as the 2nd dose after SV could induce non-inferior immunogenicity to SV3w/PZ30 regimen. Additional 5-month booster of BNT162b2 showed good immunogenicity response against Omicron variant. The short-term immune response following the booster dose was not significantly different among varying booster dose regimens of 10, 15 or 30 µg of BNT162b2.

Extended interval of primary vaccination series might be beneficial, for both higher immunogenicity and less adverse effects. For BNT162b2, anti-spike antibody level at 14–34 days after 2nd dose was higher in interval of 65–84 days, compared with 19–29 days (GM 6703 AU/ml, 95 % CI 5887–7633 vs 694 AU/ml, 95 % CI 540–893, respectively) [26]. Amirthalingam G., et al. [26] also found that vaccine effectiveness was higher in > 6-week interval of

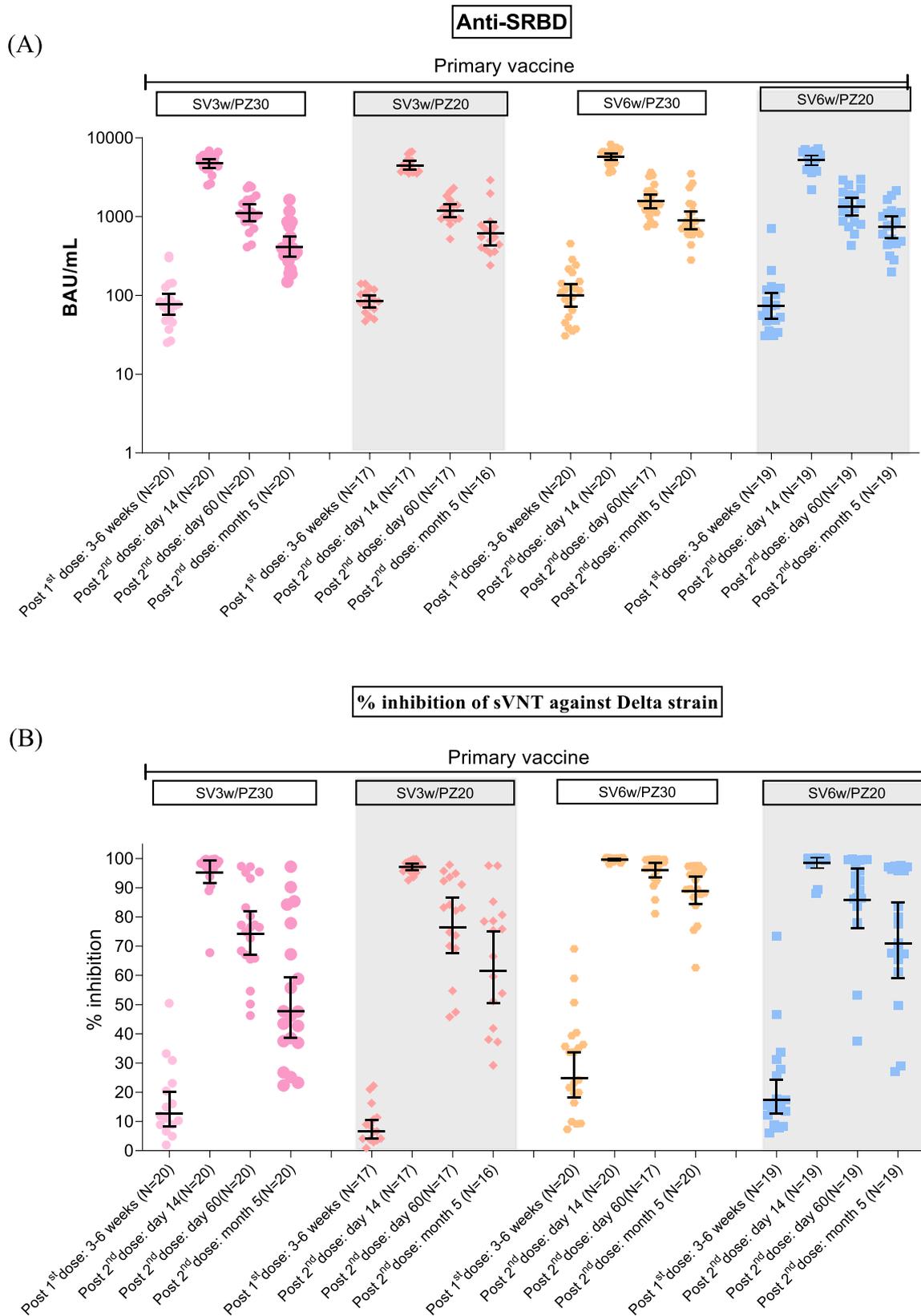


Fig. 3. Geometric means (95 % CI) of immunogenicity responses after CoronaVac/BNT162b2 vaccination as primary series in healthy adolescents by vaccination group: (A) anti-S-RBD IgG-wild type (BAU/ml), and (B) sVNT against Delta variant (%inhibition). anti-S-RBD IgG: anti-spike-receptor-binding-domain immunoglobulin G; SV3w/PZ30: 3-week-interval CoronaVac/BNT162b2 30 µg; SV3w/PZ20: 3-week-interval CoronaVac/BNT162b2 20 µg; SV6w/PZ30: 6-week-interval CoronaVac/BNT162b2 30 µg; SV6w/PZ20: 6-week-interval CoronaVac/BNT162b2 20 µg; sVNT: Surrogate virus neutralization test.

Table 2

Cell-mediated responses after CoronaVac/BNT162b2 vaccination as primary series in healthy adolescents by vaccination group.

Immunogenicity outcomes	SV3w/PZ30 (n = 10)	SV3w/PZ20 (n = 8)	SV6w/PZ30 (n = 10)	SV6w/PZ20 (n = 9)
Spike-specific T cell response (SFU/10 ⁶ PBMCs) [†]				
- Post 2 nd dose: day 14, median (IQR)	328 (200–472)	504 (400–990)	290 (232–484)	332 (176–360)
- Post 2 nd dose: day 60, median (IQR)	104 (36–192) (n = 9)	128 (88–272)	104 (60–160) (n = 9)	120 (100–136)
- Post 2 nd dose: month 5, median (IQR)	56 (22–244) (n = 8)	120 (16–400) (n = 7)	74 (38–126) (n = 8)	80 (36–100) (n = 7)
RBD-specific B cell response (SFU/10 ⁶ PBMCs) [†]				
- Post 2 nd dose: month 5, median (IQR)	25 (11–70) (n = 8)	18 (10–30) (n = 7)	8 (4–13) (n = 8)	22 (12–44) (n = 7)

PBMC: Peripheral blood mononuclear cell; RBD: Receptor binding domain; SFU: Spot forming unit; SV3w/PZ30: 3-week-interval CoronaVac/BNT162b2 30 µg; SV3w/PZ20: 3-week-interval CoronaVac/BNT162b2 20 µg; SV6w/PZ30: 6-week-interval CoronaVac/BNT162b2 30 µg; SV6w/PZ20: 6-week-interval CoronaVac/BNT162b2 20 µg;

[†] P-values comparing between vaccination arms at day 14, 60, and month 5 were 0.27, 0.73, and 0.82 for T cell response, respectively, and 0.14 for B cell response, by Kruskal-Wallis test.

BNT162b2 than the standard 3-week interval (82–94 % vs 77–88 %, respectively). Regarding the concern of myocarditis after mRNA vaccination, Buchan SA., et al. [27] showed that less myocarditis/pericarditis rate was reported in the interval of > 8 weeks than ≤ 30 days, with the rate of 11.1 per million doses (95 % CI 0.3–61.6) vs 94.5 per million doses (95 % CI 11.4–341.4) after BNT162b2 and 132.5 per million doses (95 % CI 27.3–387.2) vs 376.5 per million doses (95 % CI 102.6–964.1) after mRNA-1273 (Spikevax, Moderna) for primary vaccination series in adult from 4-week interval in registration clinical trial to optimum interval at 8 weeks was recommended by the Advisory Committee on Immunization Practices, the United States, in February 2022 [28]. Our study showed that the extended 6-week interval groups yielded higher antibody responses, including neutralizing antibody against Delta variant by sVNT and anti-S-RBD IgG-wild type, than the standard 3-week interval groups.

Less frequent and mostly mild reactogenicities was experienced after vaccination with SV than BNT162b2 as the first dose according to the previous study [2]. Adolescents in this study reported more local and systemic reactogenicities after SV than the previous report of SV in children and adolescents 3–17 years of age [6]. After BNT162b2 vaccination, the reactogenicities were similar to the previous study in adolescents [2]. Considering less reactogenicities from SV and single BNT162b2 than 2-dose BNT162b2, this vaccine regimen might be a sensible choice.

The principle of heterologous prime-boost vaccination with inactivated and mRNA vaccine has demonstrated good immunologic responses and offered a flexible regimen, especially during the period of insufficient vaccine supply. The study of adult in Thailand showed that, comparing with homologous BNT162b2 vaccination, the > 7-week-interval CoronaVac/BNT162b2 group yielded higher anti-RBD IgG [3]. Heterologous prime-boost vaccination with different vaccine antigens also has the advantage of diverse antigen delivery and potentially induce broader immune responses to SARS-CoV-2. CoronaVac was derived from inactivated whole virus of SARS-CoV-2 [1]. Thus, after vaccination, the host immune system would respond to several parts of virus e.g., spike protein, envelope, matrix, and nucleoprotein [1]. Unlike BNT162b2, which is an mRNA encoding the SARS-CoV-2 full-length spike protein vaccine, could induce high immune response to spike protein only [2].

During this period of Omicron-dominant COVID-19 pandemic, booster vaccination is needed. Data from the United States showed that vaccine effectiveness was higher after booster than 5-month post primary series [29]. Our results showed that, after 5-month BNT162b2 booster, the neutralizing antibody against Omicron

variant was enhanced, although all the used vaccines were developed from wild type virus. Centers for Disease Control and Prevention, the United States, recommended a booster dose in adolescents aged over 12 years at ≥ 5 months after primary series [30]. In Thailand, Ministry of Public Health also recommended a booster dose in adolescents aged over 12 years at 4–6 months after primary series [31]. The clinical trials on Omicron-variant vaccines booster are underway [32,33]. Meanwhile, the currently available wild-type-based COVID-19 vaccines could be used for booster.

Cell-mediated immunity has a potential role in preventing SARS-CoV-2 infection and alleviating the severity of disease [34]. After vaccination, SARS-CoV-2-specific T cell responses were demonstrated at the same time as the protective clinical effect, suggesting the pivotal role [34]. Regarding SARS-CoV-2 variants, cellular responses showed strong cross-protection [34]. The use of heterologous prime-boost regimen might extend the breadth of the cell-mediated immune responses. The robust spike-specific T cell responses were demonstrated after the inactivated and mRNA vaccine regimen in our study, with the median spike-specific T cell response of 104–128 SFU/10⁶ PBMCs at 2 months after vaccination, which was higher than post AZD1222 booster after SV primary series in adults, with the median spike-nucleoprotein-membrane protein-open reading frame proteins-specific T cell response of 80 SFU/10⁶ PBMCs at 1 month after booster, conducting in the same laboratory [21]. We demonstrated comparable T and B cell response among vaccination groups. Number of memory T and B cells were still detected at 5 months post second dose, although with lower level of B cells than T cells as reported previously [35].

The strengths of this study were the period of this trial, from October 2021 to April 2022, which included both the Delta- and Omicron-dominant era in Thailand. We reported 1-to-6-month immunogenicity results, after primary series and booster doses. The limitation was the method used to measure the neutralizing antibody. We used in-house assay instead of the gold standard microneutralization assay; however, we tested for neutralizing antibody by both sVNT and pVNT. The number of participants in this study might be too low to demonstrate the significant difference of immunogenicity between vaccine regimens, however, we provided the proof of concept of favorable extended interval in primary vaccination series. For booster sub study, as a proportion of participants declined to participate, there was the heterogeneity of participants primed with different primary vaccination series in each booster group which might influence the results. Considering logistical aspect, we proposed the use of half-dose BNT162b2 booster, which was similar to half-dose mRNA-1273 booster con-

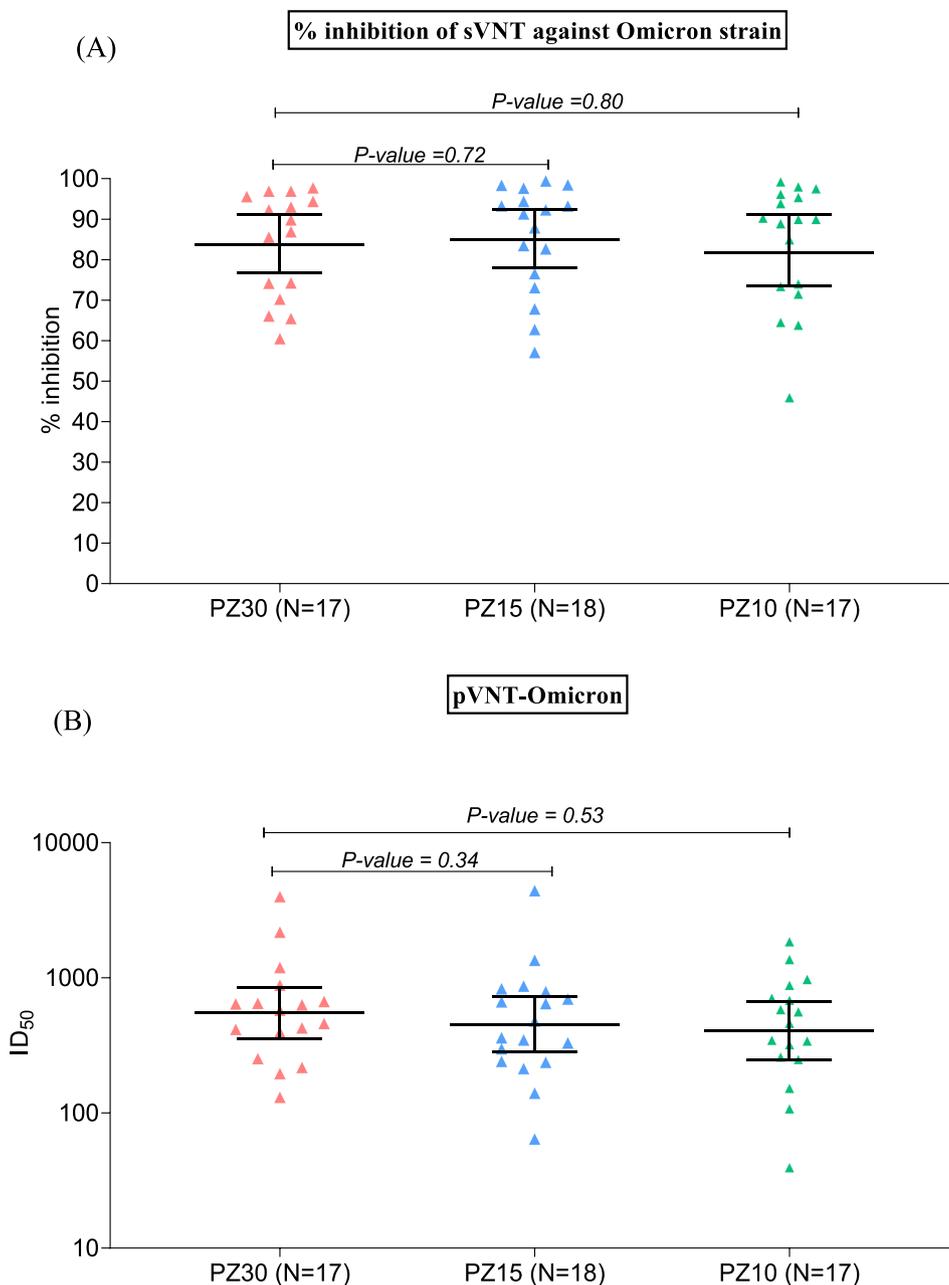


Fig. 4. Geometric means (95 % CI) of immunogenicity responses after 5-month BNT162b2 booster post CoronaVac/BNT162b2 primary series in healthy adolescents by vaccination group: (A) sVNT against Omicron variant (%inhibition), and (B) pVNT against Omicron variant (ID₅₀). ID₅₀: Neutralization dilution for 50 % pseudovirus inhibition; pVNT: Pseudovirus neutralization test; PZ10: Booster with BNT162b2 10 µg; PZ15: Booster with BNT162b2 15 µg; PZ30: Booster with BNT162b2 30 µg; sVNT: Surrogate virus neutralization test.

cept [36], while the dose of 20 µg might not be appealing for real-life implementation.

Data availability

Data will be made available on request.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jvaxc.2022.100211>.

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