Immunogenicity to SARS-CoV-2 Omicron variant among school-aged children with 2-dose of inactivated SARS-CoV-2 vaccines followed by BNT162b2 booster

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ABSTRACT

Background: A primary series of 2-dose SARS-CoV-2 vaccines based on an ancestral strain generate inadequate neutralizing antibodies against the SARS-CoV-2 Omicron variant. This study aimed to describe the immune response from giving healthy school-aged children who previously received 2 inactivated vaccines an mRNA BNT162b2 booster.

Methods: Healthy children aged 5–11 years who received 2 doses of CoronaVac or Covilo were enrolled and received 10 μg BNT162b2 intramuscularly. Neutralizing antibody against Omicron variant was measured at pre-booster and 14–21 days post-booster by surrogate virus neutralization test (sVNT, %inhibition) and pseudovirus neutralization test (pVNT, ID_{50}). Antibody responses were compared with a parallel cohort of children who received 2 doses of BNT162b2 3 weeks apart.

Results: From April to May 2022, 59 children with a mean age (SD) of 8.5 years (1.7) were enrolled: 20 CoronaVac and 39 Covilo recipients. The median interval from the primary series was 49 days (IQR 33–51). After booster, the geometric means (GMs) of sVNT and pVNT were 72.2 %inhibition (95 %CI 67.2–77.6) and 499 (95 %CI 399–624), respectively. The proportion of children with sVNT against Omicron strain ≥68 %inhibition increased from none to 70.2 %. The geometric mean ratio (GMR) of sVNT and pVNT compared with a parallel cohort were 4.3 and 12.2, respectively. The GMR of sVNT and pVNT between children who received booster dose at >6-week interval were 1.2 (95 %CI 1.1–1.3). and 1.8 (95 %CI 1.2–2.7) compared with 4–6 weeks interval.

Conclusion: A regimen of 2-dose of inactivated vaccine followed by BNT162b2 booster dose elicited high neutralizing antibody against the Omicron variants in healthy school-aged children.

1. Introduction

As of May 2022, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes Coronavirus disease 2019 or COVID-19, has infected over 530 million people worldwide, killing over 6.2 million [1]. Vaccines have become a crucial tool to control infection, reduce hospitalizations and deaths from COVID-19, and contain the pandemic. Current COVID-19 vaccine platforms that have been authorized for school-aged children, 5–11 years old, are mRNA and inactivated vaccines. Authorized mRNA SARS-CoV-2 vaccines for children 5–11 years old include BNT162b2, Comirnaty/Pfizer-BioNTech which used one-third of

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the adult dose (10 ug/dose) [2] or mRNA-1273, Spikevax/Moderna which used half of the adult dose (50 ug/dose) [3]. Inactivated SARS-CoV-2 vaccines that have received WHO prequalification, CoronaVac/Sinovac and BBIBP-CorV, Covilo/Sinopharm vaccines, manufactured by Chinese companies, are widely used in the Asia Pacific and Latin America [4].

For over two years during the SARS-CoV-2 global pandemic, the virus has continuously mutated into new variants of concern, e.g., Delta (B.1.617) and Omicron (B.1.1.529), with increasing abilities to evade immune responses elicited by SARS-CoV-2 vaccines targeting the ancestral strain. Neutralizing antibody titers against the Omicron variant are 35 to 40 times lower than against the ancestral strain [5]. In a recent study on healthy adults in Hong Kong, only 20 % of those who received two doses of BNT162b2 and none of those who were given two doses of CoronaVac had detectable neutralizing antibodies against the Omicron strain in the serum [5]. These data highlight the need for additional booster in the era of Omicron predominance. Vaccine effectiveness (VE) against hospitalization during the Omicron predominance that children 5–11 years old receive from two doses of BNT162b2 has remained at 68–74 % [6,7]. However, the protective efficacy against infection has decreased from 90 % during the Delta variant predominance [8] to 31 % during the Omicron predominant period [9]. Inactivated vaccines likewise show lower efficacy during the Omicron predominant period. A study of Covilo in children aged 3–11 years in Argentina found that VE markedly decreased from 83.4 % before the Omicron wave to 58.6 % during the Omicron period [10]. A study of CoronaVac among children 6–11 years old in Brazil reported that VE against hospitalization was only 63.5 % at ≥14 days post the second dose during the Omicron period [11].

Thus, improving immune responses to the new SARS-CoV-2 variant is important. Increasing evidence supports using heterologous booster (an mRNA vaccine booster following inactivated SARS-CoV-2 vaccines) to enhance immune response against the emerging variants of concern (VOCs) [12–14]. Zuo F, et al. found that antibodies against VOCs in adults who received the heterologous mRNA booster vaccine were similar to those who received three homologous doses of mRNA vaccine [12]. Costa Clemens SA, et al. studied in Brazilian adults and found that heterologous boosters could more strongly enhance the very low neutralizing antibody levels 6 months after receiving two doses of CoronaVac than homologous boosters: the geometric mean ratios (GMR) for heterologous BNT162b2 booster compared with homologous CoronaVac booster was 21.5 [13]. Moreover, a study on vaccine effectiveness in Chileans aged ≥16 years found that a heterologous booster with either AZD1222 or BNT162b2 offers better protection than a homologous booster with CoronaVac in those who received CoronaVac as the primary series [15]. This study aims to characterize immunogenicity and reactogenicity of BNT162b2 mRNA vaccine as a booster dose following 2 doses of inactivated vaccines in school-aged children.

2. Methods

2.1. Study design and participants

This is a single-arm phase 2 clinical trial among healthy school-aged children who received 2 doses of inactivated SARS-CoV2 vaccine. The study was conducted at 2 clinical research sites: Faculty of Medicine, Chulalongkorn University and Chonburi hospital. The inclusion criteria were (1) healthy children aged 5 to <12 years old, (2) received a 2-dose regimen of inactivated SARS-CoV-2 vaccines, either CoronaVac or Covilo, at least 28 days before enrollment. The exclusion criteria were participants who (1) had documented SARS-CoV-2 infection, (2) received immunoglobulins or blood products within 3 months, (3) received any other vaccines within 14 days (for inactivated vaccines) or 28 days (for live-attenuated vaccines) before enrollment except influenza vaccines, (4) were immunosuppressed, and (5) had anaphylaxis to any of the BNT162b2 vaccine components. Informed consent was obtained from the parent and 7- to 11-year-old participants also signed assent forms. The institutional review board of the Faculty of Medicine, Chulalongkorn University (IRB no. 0146/65) and Chonburi Provincial Hospital (IRB no 030/2565) approved this study. This study was registered in thaiclinicaltrials.org (TCTR20220330001), and was funded by the National Vaccine Institute, Thailand (2565.1/5). The comparison groups who received 2 mRNA vaccines as a primary series are from a parallel study which was approved by the institutional review board and was registered in thaiclinicaltrials.org (TCTR20220125002) [16].

Study procedure

Baseline demographics and clinical data were reviewed. After assessing for inclusion and exclusion criteria, a 5 mL blood sample was collected from each participant to evaluate baseline antibodies, followed by injecting intramuscularly 10 µg of BNT162b2 (0.2 mL), lot number FN4074 (Comirnaty/Pfizer-BioNTech, Belgium). After vaccination, participants were observed at the study site for at least 30 min and the solicited local and systemic reactogenicity were recorded by parents in the diary for 7 days. Adverse events were graded according to the Guidance for Industry Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials, 2007 [17]. Blood samples were collected at day 14–21 after the booster dose in our study and after the second dose in the parallel comparison groups to evaluate neutralizing antibodies and anti-S-RBD IgG. Participants who had a high baseline neutralizing antibody titer against the Omicron variant (B.1.1.529-BA.1), sVNT ≥68 % inhibition in accordance with the US-FDA guidelines for convalescent plasma therapy for COVID-19 [18], were suspected to have had previous natural infection, and thus were excluded from immunogenicity analysis.

2.3. Laboratory assays for SAR-CoV-2 immunogenicity

Neutralizing antibodies against SARS-CoV-2 Omicron variant were measured by two laboratory tests; surrogate virus neutralization test (sVNT) against BA.1 and pseudovirus neutralization test (pVNT) against BA.2. Serum IgG against spike protein receptor binding domain (anti-S-RBD IgG) of the ancestral strain was also measured. All tests were measured using the National Center for Genetic Engineering and Biotechnology (BIOTEC) in-house assays.

2.3.1. Surrogate virus neutralization test (sVNT)

The sVNT method was adjusted from Tan et al. [19], utilizing the recombinant S-RBD from the Omicron strain (B.1.1.529–BA.1). Briefly, the 96-well plates coated with 0.1 µg/well recombinant human ACE2 ectodomain were used to incubate the mixture of serum samples and S-RBD followed by enzyme-linked immunosorbent assay (ELISA). Pre-2019 human serum was used as the negative samples. The % inhibition was calculated as follows:

$$\%inhibition = 100 \times \left[1 - \frac{sampleOD450}{negativeOD450} \right]$$

The assay was validated with standard samples from NIBSC and other reference samples and showed <10 % CV (coefficient of variation) for mid- to high- neutralizing antibody samples and <15 %CV

for negative samples. The positive cut off value of sVNT is 30 %inhibition. However, we used sVNT against Omicron variant (B.1.1.529–BA.1) $\geq$ 68 %inhibition as a cut-off for the study. [18].

2.3.2. Pseudovirus neutralization test (pVNT)

Pseudovirus neutralization test (PVNT) against the Omicron variant was performed as described previously [20–22]. Twofold serial dilutions of the sera (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. Subsequently, suspensions of HEK293T-ACE-2 cells (2×10^4 cell/ml) were mixed with the serum-pseudovirus mixture and seeded into each well. The neutralizing antibodies were determined based on luciferase activity. Values were normalized against signals from no-serum controls. The ID_{50} values were calculated by determining the half-maximal inhibitory dilution. We used pVNT ID_{50} at 185, which correlated with 80 % vaccine efficacy against symptomatic infection in adults [23], as a cut off for the study.

2.3.3. Quantitative spike receptor binding domain IgG (anti-S-RBD IgG) ELISA

The ELISA protocol was modified from Amanat et al. [24] in which diluted serum samples were incubated in 96-well plates coated with purified recombinant Myc-His-tagged S-RBD–residues 319-541 from SARS-CoV-2 (Wuhan-Hu-1). Then, we performed ELISA. Binding-antibody units (BAU/mL), after the conversion of the OD450 with standard curve referenced from the known units of WHO standard (NIBSC 20/136), was used to report the anti-S-RBD IgG level. The assay was validated with standard samples from NIBSC and showed <10 %CV for mid- to high-IgG samples and <15 %CV for low-IgG and negative samples.

2.4. Reactogenicity

Parents recorded solicited local and systemic reactions for 7 days after the booster vaccination in a diary, and unsolicited events for 14 days after the booster vaccination until the follow up visit. The reported reactions were graded according to the Guidance for Industry Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials, 2007, into 4 grades: 1: mild symptoms that did not interfere with activities of daily life, or a fever with body temperature (BT) 38.0–38.4 °C; 2: moderate symptoms with some interference with activities of daily life, or a fever with BT 38.5–38.9 °C; 3: severe symptoms that significantly limited daily activity, or a fever with BT 39.0–40.0 °C; and 4: potentially life threatening reaction that need an emergency room visit or hospitalization, or a fever with BT >40.0 °C [17].

2.5. Statistical analysis

The sample size was calculated based on the assumption that 85 % of participants who received a BNT162b2 booster following inactivated vaccines will have sVNT against the Omicron variant $\geq$ 68 % inhibition at 14 days after booster with 10 % error and 5 % α-risk. Sample size of 49 participants was needed given that an additional 20 % of participants were estimated to drop out or develop breakthrough COVID-19 infection. Therefore, 60 children were needed for the study.

Baseline demographics, clinical characteristics, and incidence of reactogenicity following a booster dose of BNT162b were described using descriptive analysis as percentages for categorical variables and a mean (standard deviation: SD) or a median (interquartile range: IQR) for continuous variables. The Chi-squared test was used for comparison of categorical variables between two groups. The two-sample independent *t*-test was used for comparison of

immunogenicity between two groups for the GMR. Statistical significance was defined as P-value < 0.05. During follow up if a participant was diagnosed with COVID-19, the participant data was censored and not included in the immunogenicity analysis.

The primary endpoint was to determine the proportion of participants who had sVNT against Omicron variant (B.1.1.529–BA.1) $\geq$ 68 %inhibition as a cut-off for high titer antibody, adopted from the original US-FDA guide on a high titer convalescent plasma [18] 14–21 days after a booster dose. The secondary endpoints were (1) to compare the GMs of sVNT and pVNT 14–21 days after the latest vaccination in those who received the heterologous booster dose in our study with two comparison groups who had taken 2 doses of BNT162b2 at a 3-week and an 8-week interval, respectively, (2) to compare the GMs (95 % confidence interval: 95 % CI) of sVNT and pVNT against the Omicron variant, and anti-S-RBD IgG against the ancestral strain between children who were vaccinated with a primary series of Coronavac and Covilo, (3) to compare response between booster after 4–6 weeks versus >6 weeks.

Data was stored in the Research Electronic Data Capture (REDCap version 6.10.8) system [25,26]. STATA version 15.1 (Stata Corp., College Station, Texas. USA.) was used for analysis.

3. Results (600 words with 2 tables and 2 figures)

3.1. Baseline characteristics

From April to May 2022, sixty children were recruited. Fifty-nine children were eligible and enrolled with a mean age of 8.5 years (SD, 1.7). There were 20 CoronaVac and 39 Covilo recipients. Baseline characteristics of study participants are shown in Table 1. The median interval between receiving the second dose of inactivated vaccine to the booster dose was 49 days (IQR 33–51). Prior to the BNT162b2 booster, none of the participants had sVNT against Omicron variant $\geq$68 % inhibition while the GMs of anti-S-RBD IgG was 249 BAU/mL (95 %CI 215–288). During 14-days of follow-up, 2 participants had symptomatic COVID-19. Therefore, 57 participants were included for the immunogenicity analysis of post booster dose.

3.2. Immunogenicity

3.2.1. SARS-CoV-2 neutralizing antibody

On day 14 after a booster of BNT162b2, the proportion of children who had sVNT against the Omicron variant (BA.1) $\geq$ 68 % inhibition increased to 70.2 % of participants (84.2 % of CoronaVac and 63.2 % of Covilo recipients), whereas the proportion in the parallel group who received 2 doses of BNT162b2 3 weeks apart was 1.8 %, and 8 weeks apart was 35.2 % (Table 2). The GMs of sVNT against the Omicron variant (BA.1) was 72.2 % inhibition (95 %CI 67.2–77.6). The GMs of sVNT among CoronaVac recipients was 76.6 %inhibition (95 %CI 68.2–86.1), compared with 70.1 %inhibition (95 % CI 63.9–77.0) among those who received the Covilo (Table 2 and Fig. 1A). The GMR between Covilo and CoronaVac recipients was 0.92 (95 %CI 0.79–1.07). The GMR of sVNT between those who received 2-dose inactivated vaccine, CoronaVac or Covilo, followed by a BNT162b2 and those who received 2-dose BNT162b2 with a 3-week interval was 4.3 (95 % CI 3.3–5.6). The GMR of sVNT between CoronaVac recipients compared with the 2-dose BNT162b2 with 3-week interval recipients was 4.6 (95 %CI 3.2–6.5) and the GMR of sVNT between Covilo recipients compared with 2-dose BNT162b2 with 3-week interval recipients was 4.2 (95 %CI 3.2–5.6). Moreover, the GMs of sVNT were significantly higher than those who received 2 doses of BNT162b2 with an extended 8-week interval regimen, P-value 0.01.

Table 1
Baseline characteristics of school-aged children who received primary series of SARS-CoV-2 inactivated vaccine.

	Total* (n = 59)	2CoronaVac/ PZ (n = 20)	2Covilo/PZ (n = 39)	PZ/PZ (3 weeks) (n = 56)	PZ/PZ (8 weeks) (n = 54)
Age (years), mean (SD)	8.5 (1.7)	7.9 (1.8)	8.8 (1.5)	8.4 (1.8)	8.9 (1.8)
Male, N (%)	35 (59.3)	12 (60)	23 (59)	33 (58.9)	22 (40.7)
Duration from 2nd dose of vaccine (days), median (IQR)	49 (33–51)	33 (31–49)	49 (41–71)	N/A	N/A
Anti-S-RBD IgG against the ancestral strain (BAU/mL), GMs (95 %CI)	249 (215–288)	316 (269–370)	221 (181–270)	N/A	N/A

* Total of the inactivated vaccine groups (2CoronaVac/PZ and 2Covilo/PZ).
PZ = BNT162b2 (Comirnaty, Pfizer-BioNTech), anti-S-RBD IgG = IgG against spike protein receptor binding domain, sVNT = surrogate virus neutralization test, SD = standard deviation, IQR = interquartile range.

Table 2
Immune responses in school-aged children at 14 days after a BNT162b2 booster following the primary series with SARS-CoV-2 inactivated vaccine and after the second dose of BNT162b2 of the parallel group.

Immunogenicity outcomes	Total* (n = 57)	2CoronaVac/PZ (n = 19)	2Covilo/PZ (n = 38)	PZ/PZ (8 weeks) (n = 54)	PZ/PZ (3 weeks) (n = 56)
sVNT against the Omicron variant (BA.1) $\geq$ 68 %, N (%)	40 (70.2)	16 (84.2)	24 (63.2)	19 (35.2)	1 (1.8)
sVNT against the Omicron variant (BA.1), %inhibition					
GMs (95 %CI)	72.2 (67.2–77.6)	76.6 (68.2–86.1)	70.1 (63.9–77.0)	54.0 (47.6–61.0)	16.7 (11.7–23.8)
GMR	4.3 (3.3–5.6)	4.6 (3.2–6.5)	4.2 (3.2–5.6)	3.2 (2.5–4.2)	Ref
pVNT against the Omicron variant (BA.2), ID$_{50}$					
GMs (95 %CI)	499 (399–624)	497 (360–685)	502 (357–707)	254 (205–313)	41 (25–68)
GMR	12.2 (7.6–19.7)	12.2 (6.7–22.0)	12.3 (6.9–22.0)	6.2 (4.0–9.7)	ref
Anti-S-RBD IgG against the ancestral strain, BAU/mL					
GMs (95 %CI)	2381 (2192–2587)	2480 (2177–2827)	2333 (2091–2602)	2119 (1900–2364)	2242 (2041–2463)
GMR	1.1 (0.9–1.2)	1.1 (0.9–1.3)	1.0 (0.9–1.2)	1.0 (0.8–1.1)	ref

* Total of the inactivated vaccine groups (2CoronaVac/PZ and 2Covilo/PZ).
PZ = BNT162b2 (Comirnaty, Pfizer-BioNTech), sVNT = surrogate virus neutralization test, anti-S-RBD IgG = IgG against spike protein receptor binding domain, pVNT = pseudovirus neutralization test, GMs = geometric means, GMR = geometric mean ratio.

The GMs of pVNT against the Omicron variant (BA.2) were 499 (95 % CI 399–624) post booster with BNT162b2, 497 (95 %CI 360–685) in CoronaVac and 502 (95 %CI 357–707) in Covilo recipients (Table 2 and Fig. 1B). The GMR between Covilo and CoronaVac recipients was 1.01 (95 %CI 0.64–1.59). Furthermore, the GMs of pVNT were higher than those who received 2 doses of BNT162b2 with the standard 3-week interval, 41 (95 % 25–68), and with an extended 8-week interval regimen, 254 (95 %CI 205–313) P-value < 0.001 and 0.003, respectively (Fig. 1B). The GMR of pVNT of the booster group compared to the parallel group, 2-dose regimen of BNT162b2 with 3-week interval recipients, was 12.2 (95 % CI 7.6–19.7). The GMR of pVNT between CoronaVac recipients compared with the parallel group was 12.2 (95 %CI 6.7–22.0) and the GMR of pVNT between Covilo recipients compared with the parallel group was 12.3 (95 %CI 6.9–22.0).

There were 23 (40 %) children who received a BNT162b2 booster dose 4–6 weeks after the second dose of inactivated vaccine, and 34 (60 %) children who received the booster >6 weeks after the second dose of inactivated vaccine. The immunogenicity in the longer interval group was significantly higher than in the short interval group. GMs of sVNT were 64.5 (95 %CI 56.6–73.5) in the shorter interval group and 77.9 (95 %CI 72.1–84.3) in the longer interval group, GMR 1.2 (95 %CI 1.1–1.3). The GMs of pVNT were 364 (95 %CI 255–520) and 645 (95 %CI 509–841) between shorter and longer interval groups, respectively, with GMR 1.8 (95 %CI 1.2–2.7).

3.2.2. Quantitative spike receptor binding domain IgG (anti-S-RBD IgG) ELISA

On day 14 after receiving the BNT162b2 booster, participants had GMs of anti-S-RBD IgG against the ancestral strain equal to 2381 BAU/mL (95 % CI 2192–2587). There was no difference in the GMs between participants who received BNT162b2 as a booster dose following 2 doses of inactivated SARS-CoV-2 vaccine and those who received the regimen of 2-dose BNT162b2 3 weeks apart, GMR 1.1 (95 % CI 0.9–1.2) (Table 2).

3.3. Reactogenicity

After participants received the BNT162b2 booster, information on any solicited reactogenicities that occurred was collected, as shown in Fig. 2 and Supplementary Table 1. The most common local solicited reaction was pain at injection sites, found in 52.5 % (31 of 59) of participants (mild symptoms 42.4 % and moderate symptoms 10.2 %). The three most reported systemic reactions were myalgia, headache, and fatigue, which were reported by 18.6 %, 15.2 % and 13.6 % of participants, respectively. No serious adverse events were reported.

(1A)
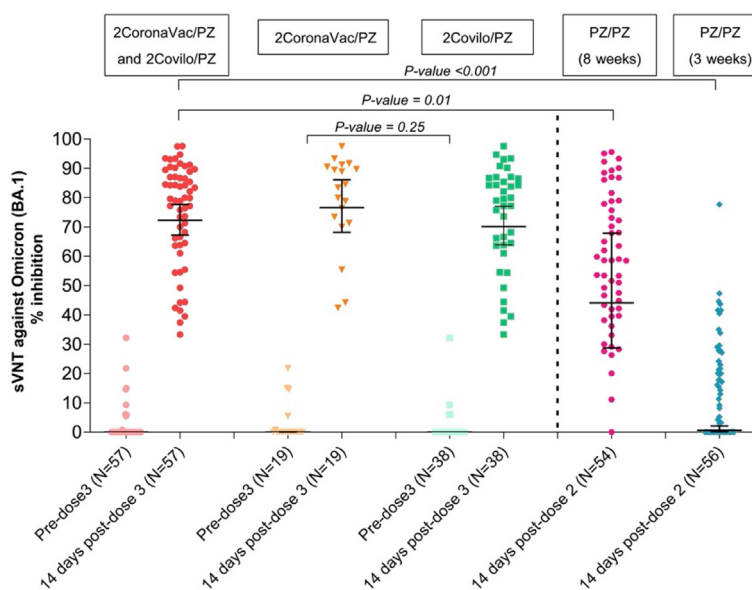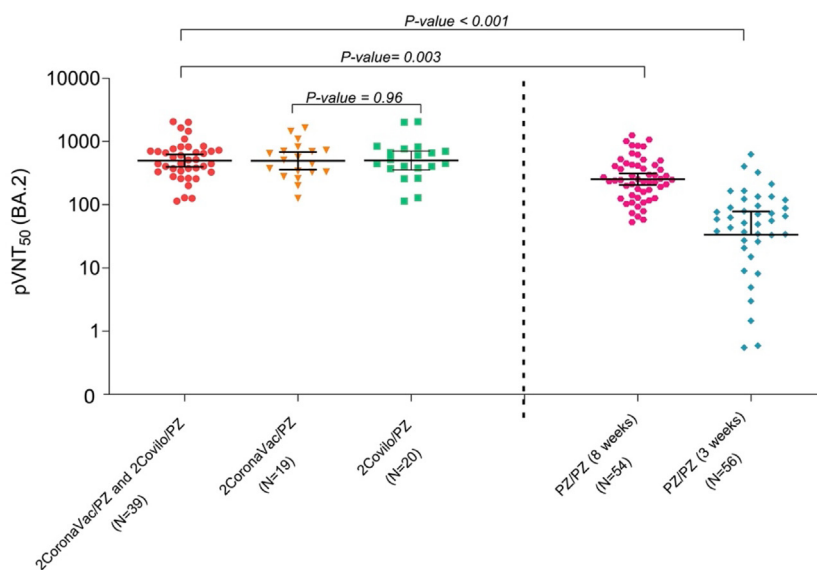

(1B)

Fig. 1. GMs of sVNT against the Omicron variant (BA.1) (**A**) and GMs of pVNT against the Omicron variant (BA.2) (**B**) at day 14 after a BNT162b2 booster following the primary series with SARS-CoV-2 inactivated vaccine among school-aged children. *P-value* from *t*-test; PZ = BNT162b2 (Comirnaty, Pfizer-BioNTech), sVNT = surrogate virus neutralization test, pVNT = pseudovirus neutralization test, GMs = geometric means.

4. Discussion

In this study on healthy school-aged children, we demonstrated that a regimen of 2 doses of inactivated vaccines followed by a BNT162b2 booster elicited high neutralizing antibody against the Omicron variants. At 14 days after booster, two-thirds of participants have sVNT against the Omicron variant ≥68 % inhibition, which was higher than those who received 2 doses of BNT162b2. Only 1.8 % of participants in the standard 3-week interval group and 35.2 % of participants in the extended 8-week interval group achieved ≥68 % inhibition. According to the national COVID-19 vaccine rollout program in Thailand, an extended 8-week interval regimen was mainly distributed.

Inactivated vaccines were distributed to children in Latin America and Asia Pacific including Thailand [4]. However, data on the immunogenicity of a booster vaccine after inactivated vaccines during the Omicron period in children is scarce. We found that neutralizing antibodies against the SARS-CoV-2 Omicron variant were higher in participants who received the heterologous booster (BNT162b2 booster after inactivated vaccines as the primary series) than in those who received 2 doses of the BNT162b2 vaccine as the primary series. After receiving the BNT162b2 booster, participants had GMs of sVNT against the Omicron variant at 72.2 % inhibition. This is consistent with studies conducted in Thailand. A study by Jantarabenjakul W, et al. on healthy adults who received the BNT162b2 booster a median of 4 months after a 2-dose regi-
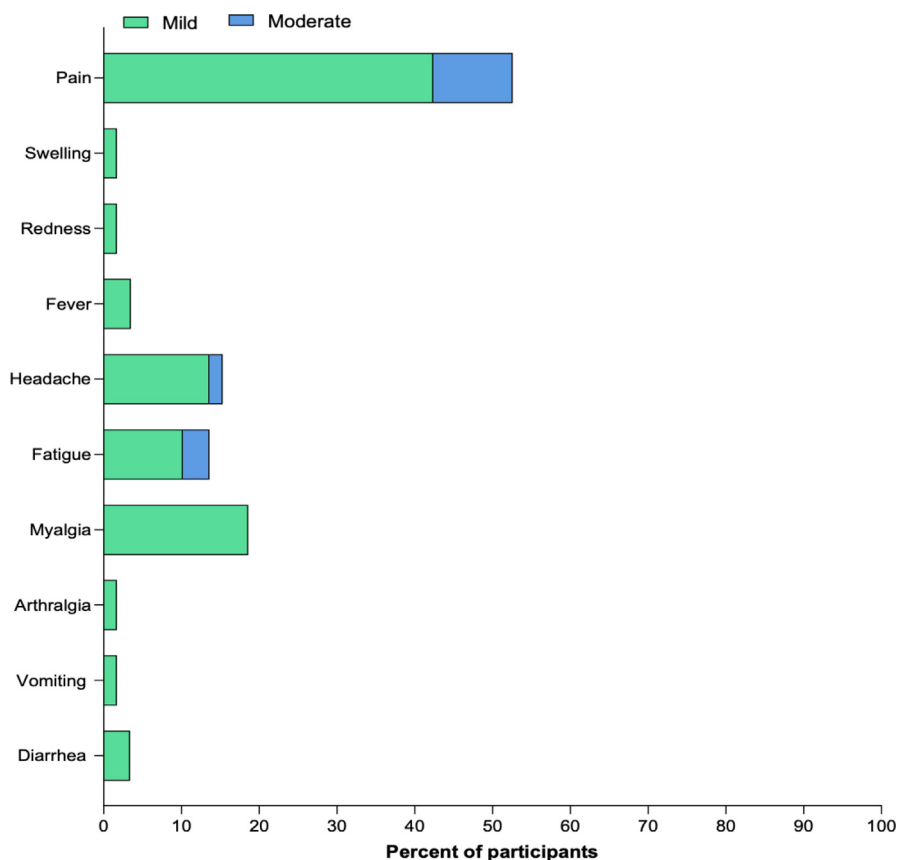
Fig. 2. Local and systemic reactogenicities within 7 days after a BNT162b2 booster in children.

men of CoronaVac, with a longer interval than our study of 7 weeks, demonstrated that participants had GMs of sVNT against the Omicron variant of 80.9 % inhibition [27]. Another study by Assawakosri S, et al. on Thai adults who received 2 doses of CoronaVac 6 months prior, found that the seroprotective rate of sVNT against the Omicron variant, cut off 30 %inhibition, increased from none to 70 % after a heterologous booster with BNT162b2. In contrast, we found that all participants in our study achieved the 30 % inhibition cut off after a BNT162b2 booster. In addition, they also found that the longer 6-month interval between the second vaccination to a booster could enhance higher immune response than the shorter 3-month interval [28]. In this study, we also observe a trend of higher immune responses among participants who received a booster >6 weeks than 4–6 weeks after primary series, GMR of pVNT 1.8, that might be the result of immunological memory response. Therefore, we support that the longer interval, at least 6 weeks, between the second and the third dose could stimulate a better immune response.

Although we did not have a comparison group of homologous 3-dose regimen, our findings are consistent with previous studies that compared the heterologous booster regimen (a mRNA vaccine following 2 doses of inactivated vaccines) with the homologous 3-dose regimen. Costa Clemens SA, et al. found that Brazilian adults who received a heterologous booster with BNT162b2 6 months after 2-dose regimen of CoronaVac could induce higher neutralizing antibodies (measured using focus reduction neutralization test (FRNT$_{50}$)) against the Omicron variant than those who received homologous 3-dose CoronaVac 28 days after booster, GMR 13 [13]. In addition, a study by Zuo F, et al. in healthy adults showed that antibodies against VOCs including the Omicron variant in participants who received the mRNA booster vaccine following 2 doses of inactivated vaccine were similar to those who received

three homologous doses of mRNA vaccine, with a median of 90 % neutralizing titer (NT90) values in both groups equal to 20. Moreover, participants who received the heterologous booster developed more S1-specific *T*-cells than those who received the homologous three doses of mRNA vaccine [12].

In this study, despite significant differences in Omicron-specific neutralizing antibodies measured using sVNT and pVNT assays between children who received an inactivated vaccine followed by booster BNT162b2 and children who received a 2-primary dose of BNT162b2, there was no difference in GMs of anti-S-RBD IgG between them. In the case of post-booster immunity, several other works have also documented this contradiction. For instance, Pérez-Then E et al. [29] reported no significant difference in levels of anti-S-RBD IgG and Plaque Reduction Neutralization Antibody Test (PRNT)$_{50}$ (ancestral strain) between vaccinees receiving 2 doses of BTN162b2 and vacciness receiving 2 doses of inactivated vaccines and a BNT162b2 booster. However, a significant difference was documented for PRNT$_{50}$ (Omicron). Similarly, Garcia-Beltran WF et al. [30] showed no difference in ID$_{50}$ (ancestral strain) between vaccinees receiving 2 doses and 3 doses of mRNA vaccines, but showed a 19- to 27-fold increase in ID$_{50}$ (Omicron) post-booster. These results are in line with our findings in children. The post-booster asymmetric increase in VOC-specific neutralizing antibodies when total anti-S, anti-S-RBD, or ancestral neutralizing antibodies are comparable remains to be investigated. Plausible explanations include affinity maturation of neutralizing antibodies or amplification of a small pool of broadly-neutralizing antibodies or antibodies that recognize alternative or minor epitopes after a booster shot [30].

In school-aged children, several studies showed reduction in vaccine efficacy (VE) of the 2-dose regimen of inactivated vaccines during the period of the Omicron variant predominance. A study of

Covilo in children aged 3–11 years in Argentina showed marked reduction of VE against hospitalization from 83.4 % during the Delta wave to only 58.6 % during Omicron predominance in 2022 [10] A study of CoronaVac among children 6–11 years old in Brazil reported that VE against hospitalization was only 63.5 % during January to April 2022, the Omicron period [11]. Likewise, studies of the of 2-dose regimen of BNT162b2, given in a 3-week interval, during the Omicron predominance in children 5–11 years old in the US found that VE against infection was 31 % [9], and against hospitalization was 74 % [6]. In May 2022, the US FDA amended the Emergency Use Authorization (EUA) to allow using BNT162b2 as a booster dose for children 5 through 11 years of age at least five months after completion of a primary series with the BNT162b2, given in a 3-week interval, which is also recommended by the US-ACIP (Advisory Committee on Immunization Practices) [31]. These data confirm that during the Omicron period, children who received a primary series of vaccines using the ancestral strain should receive an additional booster dose.

The most reported local reactogenicity in our study was pain at injection site (52.5 %), consistent with the report from a pivotal trial on reactogenicity from the first dose of BNT162b2 in children by Walter et al. with 74 % of participants reporting pain at the injection site [8]. Common systemic reactions were myalgia (18.6 %), headache (15.2 %), and fatigue (13.6 %), with higher rates of myalgia in this study than another study on children who received a first primary dose of BNT162b2 with 9 % reported myalgia while fatigue and headache were slightly lower in our study compared to the other study which reported 34 % and 22 %, respectively.

The strength of this study is that this is the phase 2 clinical trial focusing on children who were inactivated COVID-19 vaccine recipients who were then given a booster dose with BNT162b2, a topic previously lacking in data. We measured immunogenicity using neutralizing antibodies against both Omicron variants (BA.1 (sVNT) and BA.2 (pVNT), circulating VOCs during the study) and compared this with the 2-dose regimen of BNT162b2, both the standard 3-week interval and the extended 8-week interval (the main regimen in Thailand). These data strongly support the safety and immunogenicity of this heterologous booster strategy. This study has several limitations. This study was designed to evaluate safety and immunogenicity. Although, we also measured neutralizing antibodies that correlated to vaccine effectiveness [23], the correlates have only been evaluated in adults, and it remains unclear if different cut-off values would be needed in children. We did not perform a randomized control trial, nor did we have a comparison group of homologous 3-dose regimen of CoronaVac or BNT162b2 vaccines, but we used a parallel cohort of 2-dose of BNT162b2 conducted at the same clinical site and underwent immunogenicity assays at the same laboratory as a control group. There is data on the use of homologous 3-dose BNT162b2 regimen among children 5 through 11 years of age, which a booster vaccine was given at 7–9 months after the primary series. The GMT of NT50 using a focus reduction neutralization test assay against the Omicron variant increased from 27.6 at 1 month after the second dose to 614 at 1 month after a booster [32]. A preliminary data of healthy Thai children who received a homologous booster with BNT162b2 at 3 months after a primary series also showed similar immune response to this study that the GMs of sVNT against the Omicron variant was 66.5 %inhibition and the GMs of pVNT(ID$_{50}$) was 322 [33].

Our study suggests that giving children the heterologous booster with BNT162b2 can enhance neutralizing antibodies against the heavily mutated Omicron variant, the predominant circulating variant in Thailand at the time of our study. Our data supports the tolerability and immunogenicity of using the BNT162b2 as a booster dose in children who had previously received inactivated SARS-CoV-2 vaccines as the primary series, while the Omicron-specific COVID-19 vaccine is in the process of clinical development and regulatory authority approval.

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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 material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jvacx.2022.100221.

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