ORIGINAL ARTICLE

Immunogenicity of Two Doses of BNT162b2 among Children Aged 6 Months to 4 Years Following Symptomatic COVID-19

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Background: Children aged 6 months to 4 years have been recommended to receive three doses of BNT162b2, after COVID-19 infection, to immunize against SARS-CoV-2. Therefore, they might need fewer doses of vaccine compared with COVID-naïve children.

Objective: To describe the immunogenicity of 2-dose BNT162b2 following COVID-19 in healthy young children previously infected with SARS-CoV-2.

Materials and Methods: A prospective cohort study was conducted among children aged 6 months to 4 years who had SARS-CoV-2 infection during Delta-variant, which was July to November 2021, or Omicron-variant-predominant eras, which was February to August 2022. Participants received two doses of intramuscular BNT162b2 at an 8-week interval. Neutralizing antibodies against SARS-CoV-2 Omicron variant BA.4/5 were measured using pseudovirus neutralization tests (pVNT; ID_{50}) at baseline and 28 days after the second dose. Results were compared with a parallel cohort of COVID-naïve children who received 3-doses of BNT162b2 at 0, 4 and 12 weeks.

Results: Between November and December 2022, 80 children with a median age of 2.9 years (IQR 2.1 to 3.8) were enrolled. The median time from COVID-19 infection to the first dose was 13.8 months (IQR 13.8 to 16.2) in the Post-Delta Group, and 8.0 months (IQR 3.7 to 8.2) in the Post-Omicron Group. After 2-doses of BNT162b2, the geometric means (GMs) of pVNT increased from 105 (95% CI 48 to 231) to 863 (95% CI 638 to 1,168) in the Post-Delta Group and from 264 (95% CI 192 to 361) to 2,268 (95% CI 1,831 to 2,811) in the Post-Omicron Group. In comparison, the GM of pVNT was 59 (95% CI 31 to 114) in the parallel cohort of COVID-naïve children who received 3-doses of BNT162b2.

Conclusion: Two doses of BNT162b2 were able to boost the immune response with high neutralizing antibodies against the circulating Omicron variant in children who were previously infected with SARS-CoV-2.

Keywords: SARS-CoV-2 vaccine; BNT162b2; Child; Infant; Neutralizing antibody titer; Anti-SARS-CoV-2 IgG

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The coronavirus disease 2019 (COVID-19) pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) began in 2020. The impact of the pandemic on children is significant, and many of them experienced disruptions in education, socialization, and healthcare access. The SARS-CoV-2 virus has evolved over time since January 2022, when the Omicron variant (B.1.1.529) and its sublineage have become the predominant variants globally[1]. The Omicron variant has a large number of mutations within the receptor binding domain (RBD) region of the spike protein, which are related to immune escape from the SARS-CoV-2 vaccine for the ancestral strain. The presence of mutations D614G, E484A, N501Y, K417N, Y505H, and G496S increase the molecular flexibility of the S-glycoprotein to interact with the angiotensin converting enzyme 2 (ACE2) receptor, increasing the variant's infectivity[1]. In Thailand, the most prevalent strain of SARS-CoV-2 was BA.1,

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followed by variants BA.2 and BA.5 from July to October 2022, and BA.2.75 from October 2022 to February 2023[2].

COVID-19 vaccines encoding the spike protein of the SARS-CoV-2 ancestor strain were approved for children aged 6 months to 4 years by the United States Food and Drug Administration (FDA) as the final age group in mid-2022[3]. The three-dose primary series of 3-µg BNT162b2 vaccine (Pfizer-BioNTech) and two doses of the 25-µg mRNA-1273 vaccine (Moderna) were authorized for emergency use[3]. According to clinical trial data[4], vaccine efficacy was in the range of 72% to 76% among children 6 months to 4 years of age, however, this included only a short follow-up time of two months post-vaccination.

Hybrid immunity is defined as immune protection in individuals who have had at least one SARS-CoV-2 infection before or after receiving the COVID-19 vaccine[4]. Because hybrid immunity produces higher immunity than vaccination or infection alone, children with COVID-19 infection may need lesser doses of vaccines. Throughout the COVID-19 pandemic, the seroprevalence of SARS-CoV-2 infection was high in the young age group in the United States[5]. The seroprevalence was 68% in children aged less than five years in February 2022, prior to vaccine[5]. Thus, fewer doses of COVID-19 vaccines might be required[6].

Immunological studies of SARS-CoV-2 infection indicated that memory B and T cell responses appeared to persist up to 8 months after infection[7]. Studies showed that hybrid immunity leads to stronger and longer-lasting protection against the SARS-CoV-2 virus than natural immunity[8,9]. Nordström et al.[9] found that adults with hybrid immunity had a 66% lower risk of SARS-CoV-2 reinfection and 90% lower risk of COVID-19 hospitalization than adults with previous infection alone. Similarly, in children 5 to 11 years of age, the effectiveness of hybrid immunity, vaccination alone, and previous infection alone against Omicron infection was 79.4%, 15.5%, and 62.9% at four months, respectively[10].

Children were recommended to receive COVID-19 vaccines regardless of their history of SARS-CoV-2 infection. The hypothesis was that children primed with natural infection followed by the administration of fewer doses of the BNT162b2 could achieve an immune response similar to naïve children who receive a three-dose primary series of vaccines. Furthermore, the 2-dose BNT162b2 regimen would be able to boost an adequate immune response in children previously infected with SARS-CoV-2, regardless of the previously infecting variants of the virus, such as Delta or Omicron variants. Fewer dosages of the vaccine might be beneficial for improving complete vaccine schedules and the overall vaccine cost allocated. The present study aimed to describe the immune response against the Omicron variant elicited by a two-dose regimen of the BNT162b2 in children aged 6 months to 4 years previously infected with SARS-CoV-2.

Materials and Methods

Study design and participants

The present study was a prospective cohort study. The inclusion criteria were 1) children aged 6 months to 4 years without underlying chronic disease, and 2) previous symptomatic SARS-CoV-2 infection during the Delta- or Omicron-predominant time period for at least three months prior to enrollment. The date of past SARS-CoV-2 infection was determined by interviewing the parents for the date of positive test for SARS-CoV-2, either by rapid antigen test or polymerase chain reaction, or the date of close contact with other COVID-19 cases. Participants were categorized into two groups by the predominant variant during the time of acquisition of SARS-CoV-2 infection, from July to November 2021, during the Delta-predominant period (Post-Delta Group), and from February to August 2022, during the Omicron-predominant period (Post-Omicron Group). Exclusion criteria were participants who 1) received vaccination against SARS-CoV-2 prior to enrollment, and 2) had a history of anaphylaxis to any of the vaccine components. The study was conducted at the Center of Excellence for Pediatric Infectious Diseases and Vaccines, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand. The present study was approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University (IRB No. 662/65) and registered in the Thai Clinical Trials Registry (thaiclinicaltrials.org, TCTR20221018008). Informed consent was obtained from parents before conducting any study procedures. Data from the parallel study[6] among healthy young children in the same age group with no previous SARS-CoV-2 infection who received a 3-dose regimen of BNT162b2 was used as a comparison group (COVID-naïve). In brief, COVID-naïve had 29 participants and the median age was 2.8 years (IQR 1.3 to 3.6). This parallel study was conducted using the same research center and using laboratory assays to determine SAR-CoV-2 titers.

Study procedures

At the enrollment site, blood samples were collected from all participants to test for baseline immunity against the SARS-CoV-2 Omicron variant. Participants were then vaccinated with 3-µg BNT162b2 in 0.2 mL, lot number GE0695 and GG3683, intramuscularly in the deltoid muscle in children aged 2 to 4 years or in the anterolateral thigh in children aged under two years. Two doses of the BNT162b2 vaccine were given at an 8-week interval. Immunogenicity was assessed at 28 days after the second dose of BNT162b2 in both groups by measuring the pseudovirus neutralization test (pVNT), surrogate virus neutralization test (sVNT), and anti-spike receptor binding domains (S-RBD). For COVID-naïve children in the parallel study[6], the immunogenicity results were retrieved at baseline and 28 days after the third dose of BNT162b2 for comparison.

Laboratory assays for SAR-CoV-2 Immunogenicity

All assays were performed using the National Center for Genetic Engineering and Biotechnology (BIOTEC) in-house assays. Detailed methods for these assays have previously been described[6] and were briefly described here.

1) pVNT against SARS-CoV-2 Omicron variant BA.4/5 (pVNT-BA.4/5)

The pVNT against the Omicron variants was performed as described previously[11]. Two-fold serial dilutions of serum samples (starting 1:40 or 1:80) were incubated with pseudoviruses displaying the Omicron BA.4/5 spikes in a 1:1 vol/vol ratio in a 96-well culture plate for 1 hour 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 hours, neutralizing antibodies were determined based on luciferase activity following entry of the pseudovirus. Values were normalized against signals from no-serum controls. The ID_{50} values were calculated by determining the half-maximal inhibitory dilution. The limit of detection (LOD) for the pVNT assay was 40, with a 1 to 40 dilution. The pVNT assay was also standardized with the World Health Organization (WHO) reference standard (NIBSC 20/136). Precision was tested with a negative serum, a standard low positive serum (NIBSC 20/140), and a standard high positive serum (NIBSC 21/234). The %CV for intra-assay variation was lower than 20% for the low positive standard and lower than 10% for the high positive standard. The authors used pVNT ID_{50} at 185 as a cutoff for correlation with 80% vaccine efficacy, which was derived from ChAdOx1 nCoV-19 vaccine (Oxford/AstraZeneca) correlates of protection study[12].

2) sVNT against SARS-CoV-2 Omicron variant BA.4/5 (sVNT-BA.4/5)

The sVNT was adjusted from Tan et al. (2020)[13] and performed as described previously[14,15], utilizing the HRP-tagged recombinant SRBD from Omicron (BA.4/5) strain. The 96-well plates coated with 0.1 µg/well purified recombinant human ACE2 ectodomain were used to incubate with serum samples, at a 1 to 10 dilution - SRBD mixture. Then, enzyme-linked immunosorbent assay (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. Purified Human IgG at 50 µg/mL was used as the negative sample. Two dilutions of "OM1" were used as the positive controls. "OM1" collected from an individual infected with the Omicron strain of SARS-CoV-2 and tested at more than 40,000 AU/mL by SARS-CoV-2 IgG II Quant (Abbott) and more than 1,000 ID_{50} by pVNT-BA.4/5, was designated the standard Omicron serum for both the quantitative anti-S-RBD IgG-BA.4/5 ELISA and sVNT-BA.4/5 assays. The percentage inhibition was calculated as follows:

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

3) Quantitative S-RBD IgG against Omicron variant BA.4/5 (anti-S-RBD IgG-BA.4/5) ELISA

The ELISA protocol was modified from Amanat et al.[16] and performed as described previously[14,15]. Briefly, the ELISA plates were coated with purified recombinant Myc-His-tagged S-RBD, residues 319-541 from SARS-CoV-2 Omicron sublineage BA.4/5 (produced in-house from transfection in HEK 293T cells at the Virology and Cell Technology Lab, BIOTEC, Thailand). Participants' sera were diluted at 1 to 1,000 in PBS-Tween 20 buffer containing 2.5% skim milk and used as primary antibodies. HRP-conjugated human IgG was used as a secondary antibody (anti-human IgG-HRP cat. No. A8667, Sigma). After the addition of TMB substrate, OD450 was measured for each sample and converted into arbitrary units (AU/mL) of anti-S-RBD IgG-BA.4/5, using the standard curve prepared from dilutions of the standard Omicron serum (OM1, positive serum from an individual infected with the Omicron strain). The dilutions used to construct the standard curve (500-15.6 AU/mL) span the linear range of OD450

Table 1. Demographic characteristics of participants

	Total (n=80)	Post-Delta Group (n=30)	Post-Omicron Group (n=50)	COVID-naïve† (n=29)	p-value
Age (years); median (IQR)	3.0 (2.3 to 3.9)	3.2 (2.4 to 4.3)	2.7 (2.2 to 4.3)	2.8 (1.3 to 3.8)	0.05
Girl, n (%)	44 (55)	18 (60)	26 (52)	13 (45)	0.51
Time since COVID-19 (months); median (IQR)	10.6 (4.2 to 14.7)	13.83 (13.8 to 16.2)	8.0 (3.7 to 8.2)	-	-

IQR=interquartile range

† The results of COVID-naïve group derived from Nantanee R, et al. Immunogenicity of BNT162b2 in children 6 months to under 5 years of age with previous SARS-CoV-2 infection, in the era of Omicron predominance. Vaccine X 2023;15:100367[6].

measurements similar to those in anti-S-RBD IgG wild-type ELISA using the WHO international standard (NIBSC 20/136). The positive cutoff for anti-S-RBD IgG-BA.4/5 ELISA was 92 AU/mL. The cutoff value was based on the average and standard deviation of 121 negative samples collected before the COVID-19 pandemic, with values range from 2 to 127 AU/mL.

Statistical analysis

The sample size was calculated using a non-inferiority criterion for the geometric mean ratio (GMR) of pVNT-BA.4/5 after 2 doses of BNT162b2 vaccine in the Post-Omicron Group compared to children in the Post-Delta Group as the reference. Assuming a 0.4 non-inferiority margin, 90% power, 0.85 GMR, and a ratio of 1.5 to l, a minimum of 40 and 27 participants per group was required, respectively. Accounting for potential missing data, the sample size was increased by 20%, yielding a total of 80 participants. The comparison group consisted of 35 participants from the parallel study[6] who had no prior history of SARS-CoV-2 infection (COVID-naïve group).

Categorical variables were described using descriptive analysis as percentages and as a mean (standard deviation, SD) or a median (interquartile range, IQR) for continuous variables. The chi-squared test was used for the comparison of categorical variables between the two groups. The two-sample independent t-test was used for the comparison of immunogenicity between the two groups and the calculation of GMR and geometric mean fold rise (GMFR) ratio. The paired t-test was used for the comparison of immunogenicity between baseline and post-vaccination within each group and the calculation of GMFR. Statistical significance was defined as a p-value less than 0.05. Stata Statistical Software, version 18 (StataCorp LLC, College Station, TX, USA) was used for analysis.

The primary outcome was to determine the GMRs of pVNT-BA.4/5 four weeks after the second dose of BNT162b2 in children with previous SARS-CoV-2 infection in the Post-Delta Group versus the Post-Omicron Group. The secondary outcomes were 1) pVNT-BA.4/5 compared with the parallel cohort[6] of COVID-naïve children who received the 3-dose BNT162b2 regimen, 2) the levels of neutralizing antibodies against the Omicron variant BA.4/5 by sVNT and pVNT, and anti-S-RBD IgG-BA.4/5, 3) the proportion of participants who had pVNT-BA.4/5 ID_{50} at 185 or greater four weeks after the second dose of BNT162b2, and 4) the incidence of reactogenicities within seven days of receiving the first and second doses of vaccine.

Results

Study populations

From November to December 2022, 80 children were enrolled with 30 children in the Post-Delta Group and 50 children in the Post-Omicron Group (Table 1). From the parallel study[6], there were 29 participants used as a control group included in the evaluation of immunological outcomes at 28 days after three doses of BNT162b2. The median ages were 3.3 years (IQR 2.4 to 4.3), 2.7 years (IQR 2.2 to 3.8), and 2.8 years (IQR 1.3 to 3.8) in Post-Delta Group, Post-Omicron Group, and COVID-naïve Group, respectively. The number of female participants was 18 or 60%, 26 or 52%, and 16 or 45.7%, respectively. The median duration from prior infection to vaccination was 13.8 months (IQR 13.8 to 16.2) in the Post-Delta Group and 8.0 months (IQR 3.7 to 8.2) in the Post-Omicron Group. During the follow-up period, three participants were lost to follow-up, two in the Post-Delta Group and one in the Post-Omicron Group. One participant in the Post-Omicron Group was diagnosed with COVID-19 after the initial dose vaccination and excluded from the immunogenicity analysis. Therefore, 76 children, 28 in the Post-Delta Group and 48 in the Post-Omicron Group were included in the evaluation of immunological outcomes at 28 days after two doses of BNT162b2.

Table 2. Neutralizing antibody by pVNT and sVNT, and anti-S-RBD IgG against SARS-CoV-2 Omicron variant BA.4/5 after 2-dose BNT162b2 vaccination in children with history of COVID-19 and after 3-dose BNT162b2 vaccination in children without history of COVID-19

Immunogenicity outcomes	Post-Delta Group	Post-Omicron Group	COVID-naïve†
pVNT-BA.4/5 (ID_{50})	(n=30)	(n=50)	(n=35)
Baseline; GM (95% CI)	105 (48 to 231)	264 (192 to 361)	-
Baseline; GMR (95% CI)	0.37 (0.19 to 0.81)	1	-
	(n=28)	(n=48)	(n=29)
Post the 2nd/3rd dose; GM (95% CI)	863 (638 to 1,168)	2,268 (1,831 to 2,811)	59 (31 to 114)
Post the 2nd dose; GMR (95% CI)	0.38 (0.24 to 0.62)	1	-
Post the 2nd/3rd dose; GMR (95% CI)	14.57 (8.35 to 25.44)	38.30 (23.25 to 63.10)	1
sVNT-BA.4/5 (%inhibition)	(n=30)	(n=50)	(n=35)
Baseline; GM (95% CI)	42.8 (25.7 to 71.1)	35.5 (25.8 to 48.7)	0.55 (0.17 to 1.74)
Baseline; GMR (95% CI)	1.20 (0.68 to 2.17)	1	-
Baseline; GMR (95% CI)	78.06 (16.19 to 376.45)	64.76 (14.11 to 297.28)	1
	(n=28)	(n=48)	(n=29)
Post the 2nd/3rd dose; GM (95% CI)	91.2 (87.0 to 95.7)	98.7 (97.4 to 99.9)	32.9 (23.6 to 45.9)
Post the 2nd dose; GMR (95% CI)	0.92 (0.89 to 0.96)	1	-
Post the 2nd/3rd dose; GMR (95% CI)	2.77 (2.19 to 3.51)	3.0 (2.44 to 3.70)	1
Anti-S-RBD IgG-BA.4/5 (AU/mL)	(n=30)	(n=50)	(n=35)
Baseline; GM (95% CI)	51.5 (40.3 to 65.8)	48.2 (41.1 to 56.5)	32 (25.8 to 39.7)
Baseline; GMR (95% CI)	1.61 (1.19 to 2.17)	1.51 (1.15 to 1.96)	1
	(n=28)	(n=48)	(n=29)
Post the 2nd/3rd dose; GM (95% CI)	255.2 (222.1 to 293.4)	372.3 (323.3 to 428.4)	134.5 (111.6 to 162.2)
Post the 2nd dose; GMR (95% CI)	0.68 (0.55 to 0.85)	1	-
Post the 2nd/3rd dose; GMR (95% CI)	1.90 (1.49 to 2.41)	2.77 (2.24 to 3.42)	1

Anti-S-RBD IgG-BA.4/5=anti-spike-receptor-binding-domain of SARS-CoV-2 Omicron variant BA.4/5 immunoglobulin G; AU=arbitrary unit; CI=confidence interval; GM=geometric mean; GMR=geometric mean ratio; ID_{50}=neutralization dilution for 50% pseudovirus inhibition; pVNT-BA.4/5=pseudovirus neutralization test against SARS-CoV-2 Omicron variant BA.4/5; sVNT-BA.4/5=surrogate virus neutralization test against SARS-CoV-2 Omicron variant BA.4/5

† The results of COVID-naïve group derived from Nantanee R, et al. Immunogenicity of BNT162b2 in children 6 months to under 5 years of age with previous SARS-CoV-2 infection, in the era of Omicron predominance. Vaccine X 2023;15:100367[6].

Immunogenicity after BNT162b2 vaccination

Neutralizing antibody assessed by pVNT-BA.4/5

At baseline, the GM of pVNT-BA.4/5 ID_{50} in the Post-Delta Group and the Post-Omicron Group were 105 (95% CI 48 to 231) and 264 (95% CI 192 to 361), respectively, as shown in Table 2 and Figure 1. After two doses of vaccination, the GM of pVNT BA.4/5 ID_{50} was 863 (95% CI 638 to 1,168) in the Post-Delta Group and 2,268 (95% CI 1,831 to 2,811) in the Post-Omicron Group. The GM of pVNT BA.4/5 ID_{50} in the COVID-naïve Group was 59 (95% CI 31 to 114) after three doses. The GMs of pVNT BA.4/5 ID_{50} after two doses in the Post-Delta Group and the Post-Omicron Group were significantly higher than after three doses of BNT162b2 in COVID-naïve children, with GMRs of 14.57 (95% CI 8.35 to 25.44) for the Post-Delta Group and 38.30 (95% CI 23.25 to 63.10) for the Post-Omicron Group. After the second dose, the rise of pVNT BA.4/5 ID_{50} from baseline was similar in the Post-Delta Group and the Post-Omicron

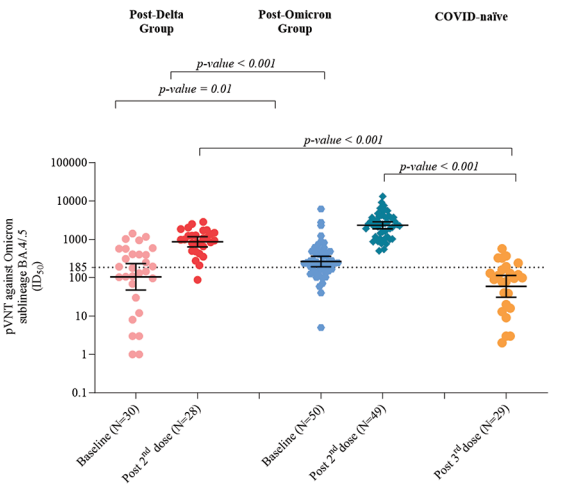

Figure 1. Geometric means (95% CI) of pVNT against Omicron variant BA.4/5 (ID_{50}) after BNT162b2 vaccination in healthy children aged 6 months to 4 years with history of COVID-19 during Delta- (Post-Delta Group), Omicron-predominant period (Post-Omicron Group) and in children without history of COVID-19.

Group with a GMFR of 8.4 (95% CI 4.2 to 17.1) and 8.7 (95% CI 6.6 to 11.3), respectively, and a GMFR ratio of 1.02 (95% CI 0.55 to 1.92).

The proportions of participants achieved pVNT-BA.4/5 ID_{50} above cutoff of 185 were 96% (27 out of 28) in the Post-Delta Group and 100% (48 out of 48) in the Post-Omicron Group. However, only 21% (6 out of 29) COVID-naïve children achieved this level after three doses of BNT162b2.

Neutralizing antibody levels by sVNT-BA.4/5

The results of sVNT-BA.4/5 are shown in Table 2 and Figure 2. Prior to vaccination, children with past SARS-CoV-2 infections had higher sVNT-BA.4/5 GMs than children without a history of SARS-CoV-2 infection, with GMRs of 78.06 (95% CI 16.19 to 376.45) for the Post-Delta Group, and 64.76 (95% CI 14.11 to 297.28) for the Post-Omicron Group. Likewise, the GMs after two doses of BNT162b2 in children with previous SARS-CoV-2 infection were significantly higher than after three doses of BNT162b2 in COVID-naïve children, with GMRs of 2.77 (95% CI 2.19 to 3.51) for the Post-Delta Group, and 3.0 (95% CI 2.44 to 3.70) for the Post-Omicron Group. The GMs of sVNT-BA.4/5 in the Post-Delta and Post-Omicron Groups were similar at baseline, with a GMR of 1.20 (95% CI 0.68 to 2.17). At four weeks after dose 2, the GM of sVNT-BA.4/5 in the Post-Delta Group was lower than that of the Post-Omicron Group, with a GMR of 0.92 (95% CI 0.89 to 0.96). Considering the increase of sVNT-BA.4/5 after two doses from baseline, both Post-Delta and Post-Omicron Groups were comparable with GMFR of 2.3 (95% CI 1.4 to 3.9) and 2.7 (95% CI 1.9 to 3.6), respectively, and GMFR ratio of 1.4 (95% CI 0.6 to 2.0).

Anti-S-RBD IgG against SARS-CoV-2 Omicron variant BA.4/5

At baseline, children with a history of COVID-19 had higher anti-S-RBD IgG-BA.4/5 than COVID-naïve children, with GMRs of 1.61 (95% CI 1.19 to 2.17) for the Post-Delta Group, and 1.51 (95% CI 1.15 to 1.96) for the Post-Omicron Group, as shown in Table 2. At four weeks after dose 2, anti-S-RBD IgG-BA.4/5 increased in both Post-Delta and Post-Omicron Groups. The antibody levels after dose 2 in children with prior COVID-19 were significantly higher than after dose 3 in COVID-naïve children, with GMRs of 1.90 (95% CI 1.49 to 2.41) for the Post-Delta Group and 2.77 (95% CI 2.24 to 3.42) for the Post-Omicron Group after dose 2.

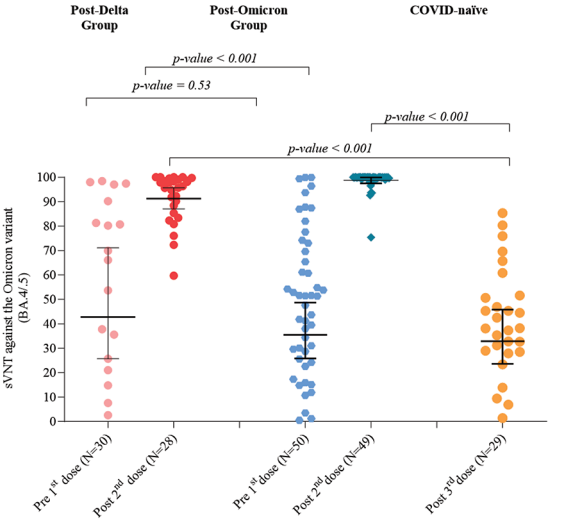

Figure 2. Geometric means (95% CI) of sVNT against Omicron variant BA.4/5 (%inhibition) after BNT162b2 vaccination in healthy children aged 6 months to 4 years with a history of COVID-19 during Delta- (Post-Delta Group), Omicron-predominant period (Post-Omicron Group) and in children without history of COVID-19.

Reactogenicity

Overall reactogenicities within seven days following the first and second doses of the BNT162b2 vaccine are shown in Table 3. Reported reactions were graded according to the age of the participants with 6 to 23 months and 2 to 4 years. Reactogenicities were mostly mild-to-moderate severity. In children aged 6 to 23 months, the most common reactogenicities were tenderness at the injection site for 18.8% and irritability for 18.8% after the first vaccination rather than after the second dose with tenderness in 13.3% and no reported irritability. In children aged 2 to 4 years, the commonly reported reactions were pain at the injection site for 10.9%, fatigue for 4.7%, and fever for 3.2% following the first dose and pain at the injection site for 1.6%, fatigue for 4.9%, and fever for 4.9% following the second dose, respectively.

Discussion

The present study found that in healthy children previously infected with SARS-CoV-2 during the Delta- or Omicron-predominant periods, two doses of BNT162b2 produced higher neutralizing antibodies against the BA.4/5 sublineage of the Omicron variant than three doses in COVID-naïve children. Robust neutralizing antibodies occurred after two doses of BNT162b2 in both Post-Delta and Post-Omicron Groups. The authors found that reactogenicities associated with the vaccine were mostly of mild or

Table 3. Solicited reactogenicity during 7 days after BNT162b2 vaccination in healthy children aged 6 months to 4 years with a history of COVID-19 during Delta- or Omicron-predominant period

Reactogenicity	Children 6 months to under 2 years of age n (%)	Children 2 to 4 years of age n (%)
BNT162b2 (1st dose)	(n=16)	(n=64)
Local reactogenicity		
• Pain		
Mild	N/A	7 (10.9)
• Tenderness		
Mild	3 (18.8)	N/A
• Redness		
Mild	1 (6.3)	-
• Swelling	-	
Mild	-	-
Systemic reactogenicity		
• Fever	-	2 (3.2)
Mild	-	1 (1.6)
Moderate	-	1 (1.6)
• Decreased appetite		
Mild	-	N/A
Moderate	1 (6.3)	N/A
• Irritability	3 (18.8)	
Mild	2 (12.5)	N/A
Moderate	1 (6.3)	N/A
• Fatigue	N/A	3 (4.7)
Mild	N/A	2 (3.1)
Moderate	N/A	1 (1.6)
• Vomiting		
Mild	-	1 (1.6)
• Diarrhea	-	2 (3.2)
Mild	-	1 (1.6)
Moderate	-	1 (1.6)
• Myalgia		2 (3.2)
Mild	N/A	1 (1.6)
Moderate	N/A	1 (1.6)
• Arthralgia		
Mild	N/A	-
BNT162b2 (1st dose)		
Systemic reactogenicity		
• Headache		
Mild	N/A	1 (1.6)
BNT162b2 (2nd dose)	(n=15)	(n=61)
Local reactogenicity		
• Pain		
Mild	N/A	1 (1.6)
Moderate	N/A	-
• Tenderness		
Mild	2 (13.3)	N/A
• Redness		
Mild	-	-
Systemic reactogenicity		
• Fever	1 (6.7)	3 (4.9)
Mild	1 (6.7)	2 (3.3)
Severe	-	1 (1.6)
• Decreased appetite		
Mild	-	N/A
• Fatigue	N/A	3 (4.9)
Mild	N/A	1 (1.6)
Moderate	N/A	2 (3.3)
• Vomiting	-	3 (4.9)
Mild	-	2 (3.3)
Moderate	-	1 (1.6)
• Diarrhea	1 (6.7)	2 (3.3)
Mild	-	2 (3.3)
Moderate	1 (6.7)	-
• Myalgia		
Mild	N/A	1 (1.6)
• Headache		
Moderate	N/A	1 (1.6)

N/A=not available

moderate severity.

Among children with prior SARS-CoV-2 infection, two doses of BNT162b2 induced higher neutralizing antibodies than the 3-dose primary series in children without prior SARS-CoV-2 infection. Thus, fewer dosages of COVID-19 vaccines could be recommended for children with prior COVID-19. The present study showed that the GMs of pVNT against Omicron variant BA.4/5 after the second dose of BNT162b2 were 14.6-fold higher in the Post-Delta Group and 38.3-fold higher in the Post-Omicron Group than in children without history of SARS-CoV-2 infection after three doses of BNT162b2. Similarly, Nantanee et al.[6] found that in children aged 6 months to 4 years who had a history of prior SARS-CoV-2 infection due to the Omicron variant receiving two doses of BNT162b2, the GMs of pVNT against BA.4/5 sublineage was 32-to-35-fold higher than in COVID-19 naïve children receiving three doses of BNT162b2. In adults, the effectiveness of hybrid immunity, previous infection, and three doses of BNT162b2, was 77%, which was higher than the effectiveness of receiving three doses of BNT162b2 alone (52%)[17]. The systematic

review and meta-regression also showed that hybrid immunity caused by previous SARS-CoV-2 infection and COVID-19 vaccine generated greater protection against reinfection than previous infection alone or booster vaccination alone, with the pooled relative protection of 46% to 59% and 88%, respectively[18].

Regardless of prior infection by Delta or Omicron SARS-CoV-2 variants, the rise of neutralizing antibodies against Omicron variant BA.4/5 in prior-infected children after two doses of BNT162b2 was similar. In children previously infected with either Delta or Omicron variants of SARS-CoV-2, the pVNT-BA.4/5 ID_{50} exceeded the cut-off threshold of 185 in over 96% in both Post-Delta and Post-Omicron Groups after the second dose. The GMFR ratio of pVNT-BA.4/5 and sVNT-BA.4/5 after two doses from baseline was 1.02 (0.55 to 1.92) and 1.4 (0.6 to 2.0) in the Post-Omicron Group compared with the Post-Delta Group, showing an insignificant difference in the rise of neutralizing antibody levels. However, Linderman et al.[19] reported the neutralizing antibody level by live virus neutralization assays against Omicron sub lineage BA.1 was higher in vaccinated Omicron-infected individuals than in vaccinated Delta-infected patients, which might be due to the similarity of the infected strain and the strain used in assays.

Single-dose vaccination with updated strategy should be considered recommended for individuals with previous SARS-CoV-2 infection regardless of variants. This generates robust protection against hospitalization or death caused by reinfection[20]. In children 6 months to 4 years of age, a history of household contact with COVID-19 might be suggestive of past SARS-CoV-2 infection. The study of seroprevalence in the United States in February 2022 found that 68% of children under 5 years of age were seropositive, with documented COVID-19 infection in only 17.5% of the pediatric population[5]. Consistent trends were observed by seroprevalence in Thailand, where 83% of children under 5 had seropositive results and only 43% had documented COVID-19 infection. COVID-19 vaccination should be considered due to the waning of natural immune protection over time. In April 2023, the Royal College of Pediatricians of Thailand recommended a single-dose vaccination for children previously infected with SARS-CoV-2[21]. The Joint Committee on Vaccination and Immunisation of the United Kingdom[22] recommended a single dose of COVID-19 vaccine as a primary course vaccination in all, regardless of previous infection status.

Reactogenicities after BNT162b2 vaccination were mostly of mild or moderate severity. In children 6 months to 2 years of age, the most commonly reported reaction in the present study was tenderness at the injection site for 18.8%, which was similar to the pivotal trial at 15% to 17%[23], but irritability for 18.8% was less frequent than the prior study at 44% to 51%[23]. In children 2 to 4 years of age, the commonly reported reactions were pain at the injection site for 1.6% to 10.9% and fatigue for 4.7% to 4.9%, which were less frequent than in the pivotal trial at 27% to 31% and 24% to 30%[23]. The previous study[24] revealed the highest incidence of myocarditis occurred in adolescents aged 12 to 16 years who received two doses of BNT162b2, predominantly after the second dose. No cases of myocarditis were reported among children aged 6 months to 4 years after BNT162b2 or mRNA-1273 vaccination[24,25].

The strengths of the present study included the evaluation of immunogenicity focused on the circulating sublineage of Omicron variant BA.4/5, the circulating sublineage in Thailand during the study period, using a pseudovirus neutralization assay. Secondly, the authors studied children previously infected with both Delta and Omicron variants. However, the present study had limitations due to the short follow-up period. Only healthy children were included, so the results were not generalizable to other populations, such as immunocompromised children. Given constantly changing circulating strains, cautious interpretation of the results is needed. In Thailand, XBB sublineage have become the predominant circulating strains since March 2023[2]. For autumn 2023, monovalent XBB booster dose was recommended by the United States FDA[26] and the European Medicines Agency[27]. Instead of using the microneutralization assay to measure immunogenicity, the authors' laboratory team used pVNT, which produced similar results in previous studies[28,29].

Conclusion

Two-dose BNT162b2 induced higher neutralizing antibodies against the circulating Omicron variant in children previously infected with SARS-CoV-2 than in COVID-naïve children. Therefore, a lower dosage of the vaccination against COVID-19 infection among them should be done according to the concept of hybrid immunity. This approach might be beneficial to reduce overall cost allocated for vaccination.

What is already known on this topic?

Hybrid immunity produces higher immunity against SARS-CoV-2 than vaccination or infection alone in children, adolescents, and adults.

What does this study add?

In children previously infected with SARS-CoV-2 during the Delta- or Omicron-predominant period, two doses of BNT162b2 produced higher neutralizing antibodies against the BA.4/5 sublineage of the Omicron variant than three doses in naïve children. Robust neutralizing antibodies occurred after two doses of BNT162b2 in both Post-Delta and Post-Omicron Groups.

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Authors' contributions

SP, NC, RN, SA, TM, TJ, and TP contributed to the conception and design of this study and the acquisition of the data. SP, NC, OH, RN, SA, JS, and TP analyzed and interpreted the data. SP drafted the manuscript. NC, RN, SA, and TP contributed to the manuscript revision. All authors read and approved the final manuscript.

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Conflicts of interest

The authors declare no conflict of interest.

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