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Article

# Homologous or Heterologous COVID-19 Boosters Regimens Significantly Impact Sero-neutralization of SARS-CoV-2 Virus and Variants

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**Abstract:** We determined the levels of neutralizing antibodies against the SARS-CoV-2 ancestral strain, Delta and Omicron variants of concern (VOCs) in 125 healthcare workers, who received CoronaVac as their primary vaccination and later received either a single ChAdOx1 or a combination of two consecutive boosters using either two ChAdOx1 doses or a ChAdOx1 or BNT162b2 as the primary and second boosters, respectively, or 2 doses of BNT162b2. The titers 12 weeks after primary vaccination were inadequate to neutralize the all strains. After a single ChAdOx1 booster, the levels of neutralization at Day 30 varied significantly, only a small proportion of participants developing neutralizing titers against Omicron at Day 7 and 30. The two doses of ChAdOx1 as the booster induced lowest activity. A combination ChAdOx1 and BNT162b2 induced greater neutralization than by two doses of ChAdOx1. Two doses of BNT162b2 as the booster had the maximal activity against Omicron VOC.

**Keywords:** COVID-19; vaccine; booster; variants of concern; neutralization

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## 1. Introduction

Starting in 2020, the availability of COVID-19 vaccines has marked the most significant effort to control Coronavirus disease (COVID-19) and reduce severe acute respiratory syndrome coronavirus (SARS-CoV-2) infections [1]. Evidence demonstrates the effectiveness of COVID-19 vaccines to prevent infection and severity of COVID-19 [2, 3]. However, vaccine-induced immunity wanes overtime and boosting is required for continued protection against emerging SARS-CoV-2 variants [4, 5]. We have previously reported immunity after primary vaccination with CoronaVac (Sinovac) and ChAdOx1 nCoV-19 (Vaxzevria, Oxford-AstraZeneca) vaccines [6, 7]. With the emergence of the Alpha, Beta, Delta, Gamma, and Omicron variants of concern (VOCs), as recognized by the World Health Organization (WHO), vaccine boosters are essential to maintain adequate immunity. Studies into the extent and durability of vaccine-induced neutralizing

antibody levels are very important to identify optimal boosting strategies. Approaches for booster application are diverse due to the availability of different vaccine platforms in each country. Homologous or heterologous COVID-19 prime-boost vaccinations have been reported to improve humoral and cellular immune responses. A homologous third dose of CoronaVac (an inactivated SARS-CoV-2 vaccine, Sinovac Life Science) demonstrated increased immune responses against SARS-CoV-2; however, immunity was lower when compared to heterologous boosting with BNT162b2 [8]. The Beta, Delta, and Omicron VOCs have shown the ability to evade immune responses induced by vaccination or prior infection [9-11]. Previous studies have shown a decrease in booster-derived immunity protection against Delta and Omicron VOCs [12, 13]. Additional studies are needed to dissect antibody levels after a second booster. A 4th dose of BNT162b2 (Pfizer–BioNTech) or mRNA-1273 (Moderna) have been shown to induce IgG antibodies against SARS-CoV-2 receptor-binding domain (RBD) and increase neutralizing antibody titers to levels higher than in unboosted individuals. In addition, the rates of SARS-CoV-2 infection and severe COVID-19 were lower after a fourth dose of BNT162b vaccine, underscoring the importance of boosters against Omicron infection, especially among those with high risk for severe COVID-19 [14]. CoronaVac, an inactivated COVID-19 vaccine, is widely used in many countries, which have adopted this vaccine for primary prevention. Little data exist describing booster-derived immunogenicity against VOCs like Delta and Omicron in recipients of CoronaVac as primary vaccination. In this study, we determined the levels of neutralizing antibodies by microneutralization assays against the ancestral strain of SARS-CoV-2 and the Delta and Omicron VOCs in COVID-19 vaccinated healthcare workers (HCWs), who received CoronaVac as their primary vaccination and later received either a single ChAdOx1 booster or a combination of two consecutive boosters using either two ChAdOx1 doses or a ChAdOx1 or BNT162b2 as the primary and second boosters, respectively, or 2 doses of BNT162b2.

## 2. Materials and Methods

### 2.1. Cell line and virus

Vero E6, green monkey kidney epithelial cell line was obtained from ATCC. Cells were grown in Eagle's minimum essential medium (EMEM, Invitrogen, US) supplement with 5% heat-inactivated fetal bovine serum (HIFBS, Invitrogen, US) 1% L-glutamine, 1% P&S, 40 µg/ml gentamicin and 0.25 µg/ml fungizone, at 35±2 °C, 5% CO<sub>2</sub> incubator. One-day-old cells were used for measuring of neutralizing antibody by microneutralization assay.

SARS-CoV-2 viruses, ancestral lineage (isolate Hong Kong/VM20001061/2020, NR-52282) was obtained through BEI Resources (NIAID, USA). The Delta B.1.617.2 (hCoV-19/Thailand/CU-A21287-NT/2021) and Omicron BA.1 (hCoV-19/Thailand/CU-A211051-NPS/2022) variants were isolated from clinical specimens collected at King Chulalongkorn Memorial Hospital. All isolates were propagated in Vero E6 cells to generate sufficient titers (100TCID<sub>50</sub>) for the microneutralization assay. All isolates were quantitated by tissue culture infectious dose 50 (TCID<sub>50</sub>) using the Reed-Muench method based on eight replicates per titration.

### 2.2. Microneutralization assay

Microneutralization (MN) assay was used to determine neutralizing (NT) antibodies against SARS-CoV-2 viruses including the ancestral strain, B.1.617.2 (Delta) and BA.1 (Omicron) variants [15]. All procedures were performed in a BSL-3 laboratory, following a standard neutralization assay using cytopathic effect (CPE)-based colorimetric read-outs. Each assay included eight cell control (CC) and virus control (VC) wells. Serum samples were diluted with 2% HIFBS/EMEM media (Invitrogen, US) at 1: 10 dilutions before heat-inactivation at 56 °C for 30 minutes. An equal volume of diluted serum was separately incubated with 100 TCID<sub>50</sub> of SARS-CoV-2 virus in 37 °C, 5% CO<sub>2</sub> incubator for 1 hour. The 100 µl of serum-virus mixture was inoculated into duplicate wells of

VeroE6 cells in 96-well plates and incubated at 37°C, 5% CO<sub>2</sub> for 5 days before staining with 0.02% neutral red (Sigma, US) in 1X PBS (Invitrogen). After an additional incubation at RT for 1 hour, lysis solution was added before measuring OD at 540 nm. Percentage of virus infectivity in VC and samples were calculated based on OD of CC, Infectivity (%) = (OD of CC – OD of sample) × 100. The percentage of inhibition was calculated using the following formula, Inhibition (%) = 100 – [(100 × Infectivity of sample)/Infectivity of VC]. Percentage of inhibition at equal or higher than 50% (≥ 50% inhibition) is considered as positive cut off for neutralizing antibody against SARS-CoV-2 strain or variant.

### 2.3. Statistical analysis

Data analysis was performed using R program Version 4.0.2 and GraphPad version 9.0 (GraphPad Software, Inc., USA). Paired t-test, Mann-Whitney U test or Wilcoxon test was used to compare the mean of % inhibition obtained against different SARS-CoV-2 variants and the differences between base line and post-boost sera. A *p*-value < 0.05 was considered statistically significant.

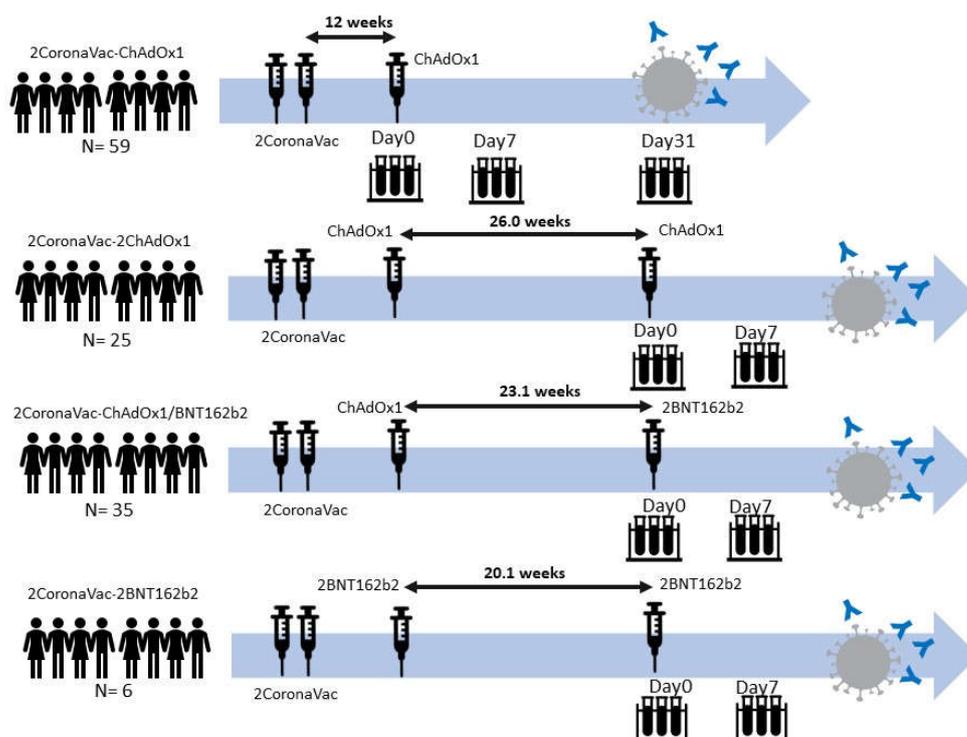
## 3. Results

### 3.1. Demographic data of participants

A total of 125 health care workers were enrolled in this study, all of which completed primary COVID-19 vaccination with CoronaVac (two doses). Table 1 and 2 list the demographic characteristics of all subjects. Subjects were divided into 4 distinct groups (Figure 1). Fifty-nine (59, 2CoronaVac-1ChAdOx1, Table 1) subjects received a single ChAdOx1 booster 13 weeks after their primary vaccination. Blood samples were collected at day 0, 1 week and 4 weeks after receiving the booster dose. Blood samples were used to measure neutralizing antibodies. This group did not receive a second booster during this study. Twenty-five (25, 2CoronaVac-2ChAdOx1, Table 2) participants received two consecutive ChAdOx1 boosters at approximately 13 weeks post primary vaccination and 182 days (Median 26.0 weeks, IQR: 21.4–26.5) after the first booster, respectively. Blood samples were collected at day 0 and 1 week after the second booster. Thirty-five (35, 2CoronaVac-ChAdOx1/BNT162b2, Table 2) subjects received a ChAdOx1 booster (13 weeks after primary vaccinations) and a BNT162b2 as a second booster, 162 days (Median 23.1 weeks, IQR: 21.4–26.6) after. As before, blood samples were collected at day 0 and 1 week after the second booster. Six (6, 2CoronaVac-2BNT162b2, Table 2) subjects received 2 consecutive BNT162b2 boosters, approximately 12 weeks after the primary vaccinations and 141 days (Median 20.1 weeks, IQR: 20.1–20.2) after the primary booster, respectively. Again, blood samples were collected at day 0 and 1 week after the second booster. The median time between the primary vaccination and the first booster for all subjects was 14.57 (Median, IQR: 14.00–17.3) weeks and the median time between the first and the second booster was 23.43 (Median, IQR: 21.4–26.5) weeks. The outline of subjects' vaccine/booster distribution and schedule is detailed in Figure 1. Boosters for HCWs in Thailand was recommended soon after the detection of VOCs. Booster vaccinations were selected based on HCWs' preferences.

**Table 1.** Demographic data of participants who received primary vaccination by CoronaVac and ChAdOx1 as the first booster.

Number of participants	59
Age, median (years)	40
IQR	32.5–52.5
20–30 years	11
31–40 years	19
41–50 years	13
51–60 years	15
61–70 years	1
BMI, median	22.2
IQR	20.9–26.3
Range	16.1–33.2
Duration between 2CoronaVac and ChAdOx1, median (weeks)	14.1

**Figure 1.** Vaccination and booster distribution and schedules.

**Table 2.** Demographic data of participants who had received primary vaccination by CoronaVac and then had the first and second booster by 2ChAdOx1 or ChAdOx1/BNT162b2 or 2BNT162b2.

Characteristics	2CoronaVac- 2ChAdOx1	2CoronaVac- ChAdOx1/BNT162b2	2CoronaVac- 2BNT162b2	p-value
Number of participants	25	35	6	
Age, median (years)	42	35	33	
IQR	36–54	26.5–49	29.5–44	0.370
Range	22–62	23–60	28–57	
Age group, n				
20–30 years	5	11	2	
31–40 years	6	9	2	
41–50 years	6	7	1	
51–60 years	7	8	1	
61–70 years	1	0	0	
Sex (% Male)	32.0	42.9	66.7	0.283
BMI, median	21.5	24.0	25.4	
IQR	19.4–27.5	20.9–25.91	24.15–26.36	0.489
Range	16.0–33.2	16.37–30.32	21.97–27.51	
Duration between 2CoronaVac and the first booster, median (weeks)	14.5	16.7	19.3	0.0212
Duration between the first and the second booster, median (weeks)	26.0	23.1	20.1	0.0047
IQR	21.4–26.5	21.4–26.6	20.1–20.2	
Range	21.4–27.4	13.0–28.0	16.0–20.2	

### 3.2. Neutralizing antibodies against SARS-CoV-2 ancestral strain, Delta and Omicron VOCs

We determined neutralizing (NT) antibodies (both as mean percentage inhibition and NT positivity rate) against SARS-CoV-2 ancestral strain, Delta and Omicron VOCs for all groups (Figure 2A to 2C and Figure 3). A sample was reported as positive for NT antibodies if achieving viral neutralization higher than 50%. When measured against the SARS-CoV-2 ancestral strain, only 13.6% of subjects in the 2CoronaVac-1ChAdOx1 group were NT positive at the time of booster dose (Day 0, Figure 2A) and a mean percentage inhibition (MI) of 14.3% (95% CI: 7.1–21.5%). However, 98.5% of subjects were NT positive at week 1 ( $p < 0.0001$ ) with a MI of 81.9% (95% CI: 77.8–86.0%) and a 100% at week 4 post-booster, with a MI of 81.3% (95% CI: 78.2–84.4%). Ninety-six percent (96%) of the 2CoronaVac-2ChAdOx1 subjects who received a second ChAdx1 booster dose were NT positive at the time of their second booster (day 0) with a MI of 88.1% (95% CI: 82.2–92.9%). All (100%) were NT positive at week 1 post-second booster and a MI of 91.6% (95% CI: 87.5–95.7%). Similarly, 100% of the subjects in the 2CoronaVac-ChAdOx1/BNT162b2 group and in the 2CoronaVac-2BNT162b2 group were NT positive against the ancestral strain at day 0 (MI of 84.5%, 95% CI: 80.5–88.6%; and MI of 84.6%, 95% CI: 73.2–95.9%, respectively) and at 1 week (MI of 85.2%, 95% CI: 81.8–88.7%; and MI of 93.3%, 95% CI: 86.3–100.3%, respectively) after their second booster dose. Figure 2B shows NT antibody titers against the Delta VOC for all groups. In the 2CoronaVac-1ChAdOx1 group, only 8.5% of subjects were NT positive against the Delta VOC at day 0 post-booster, with a MI of 1.9% (95% CI: 0.7–3.1%), but the percentage increased significantly ( $p < 0.0001$ ) to 91.5% at week 1 post-booster with a MI of 76.8% (95% CI: 70.9–82.7%) and again to 98.1% at week 4 post-booster with a MI of 81.3% (95% CI: 76.9–

85.7%). The rates of NT positivity against the Delta VOC among the 2CoronaVac-2ChAdOx1 subjects increased from 56% with a MI of 49% (95% CI: 35.3–62.7%) at Day 0 post-second booster to 76% with a MI of 70.4% (95% CI: 58.9–81.9%,  $p = 0.005$ ) at week 1 post-second booster. Among the 2CoronaVac-ChAdOx1/BNT162b2 subjects, % rates increased from 62.9% with a MI of 56.4% (95% CI: 45.5%–67.3%) at Day 0 to 97% with a MI of 80.9% (95% CI: 76.8–85.1%) at week 1 post-second booster. One hundred percent (100%) of all subjects in the 2CoronaVac-2BNT162b2 group were NT positives against the Delta VOC at Day 0 (MI of 80.7%, 95% CI: 71.8–89.7%) and week 1 (MI of 84.1%, 95% CI: 70.7–97.5%) post-second booster. Figure 2C shows NT antibody titers against the Omicron VOC for all groups. In general, NT rates and titers against Omicron VOC were relatively lower for all groups and time points. NT positivity rate for subjects in the 2CoronaVac-1ChAdOx1at day 0 post-booster was 0% with a MI of 1.2% (95% CI: 1.0–1.3%), which increased significantly ( $p < 0.0001$ ) at week 1 to 18.6% with a MI of 21.8% (95% CI: 13.3–30.3%) and to 21.2% with a MI of 22.2% (95% CI: 12.9–31.5%) at week 4. Only 4% of subjects in the 2CoronaVac-2ChAdOx1 group were NT positive against the Omicron VOCs at Day 0 post-second booster, with a MI of 7.4% (95% CI: 0–16.0%). NT positive rates increased to 16% at week 1 with a MI of 15.1% (95% CI: 2.6–27.7%). NT positivity rate among subjects in the 2CoronaVac-ChAdOx1/BNT162b2 group went from 11.4% with a MI of 10.0% (95% CI: 0.2–19.8%) at Day 0 to 71.4% with a MI of 58.7% (95% CI: 45.4–72.0%) at week 1. NT positivity rate among subjects in the 2CoronaVac-2BNT162b2 increased from 16% with a MI of 16.0% (95% CI: 0–55.4%) at Day 0 to 100% with a MI of 73.6% (95% CI: 61.2–86.1%) at week 1. Subjects in the 2CoronaVac-ChAdOx1/BNT162b2 and 2CoronaVac-2BNT162b2 groups had significant higher MI against Omicron VOC than the CoronaVac(x2)/ChAdOx1(x2) group at week 1 post-second booster ( $p = 0.034$  and  $p < 0.0001$ , respectively).

Fig. 2A Neutralizing antibody against Ancestral strain

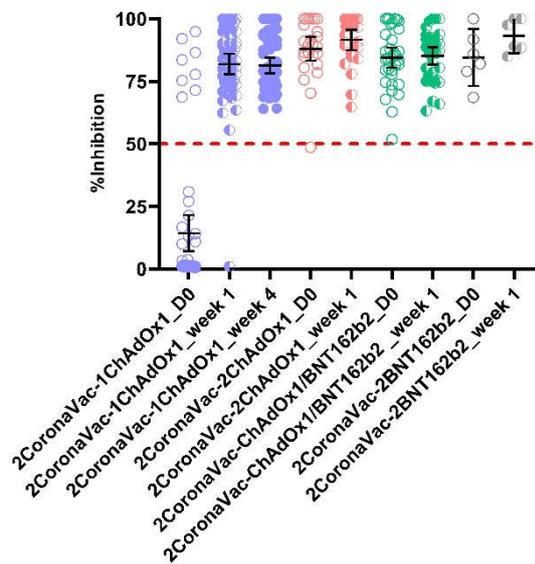


Fig. 2B Neutralizing antibody against Delta variant

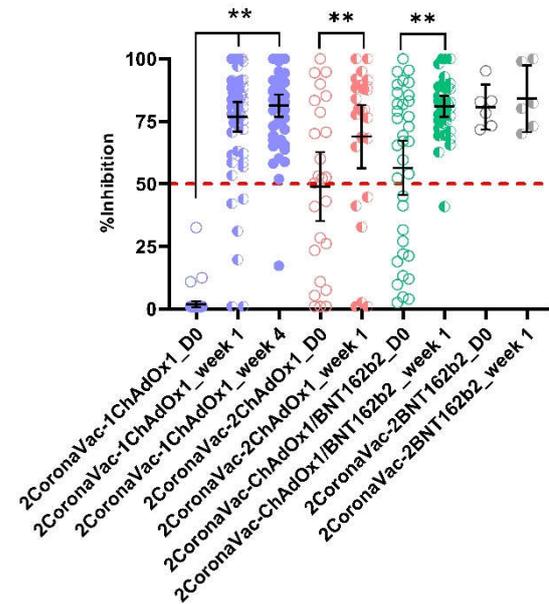


Fig. 2C Neutralizing antibody against Omicron variant

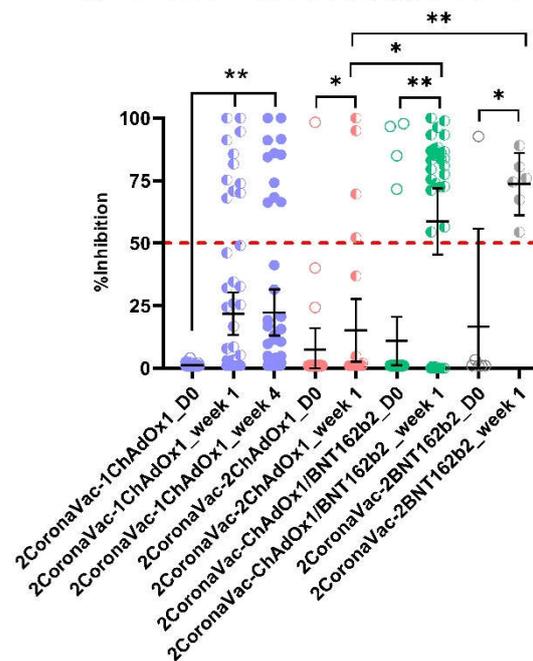
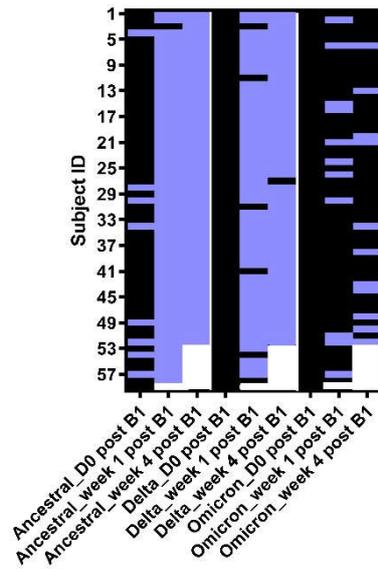
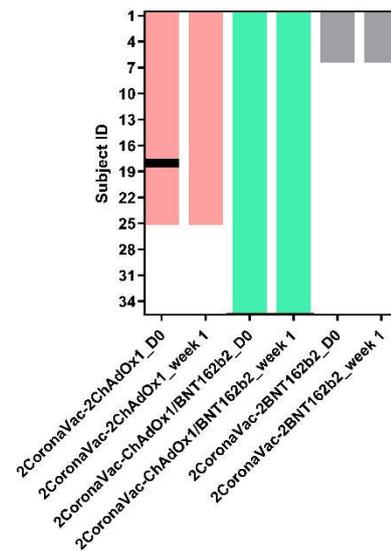


Figure 2. Neutralizing (NT) antibodies against SARS-CoV-2 Ancestral strain (2A), Delta (2B) and Omicron (2C) VOCs. A “\*” indicates significance of  $p < 0.05$ . A “\*\*” indicates significance of  $p < 0.0001$ .

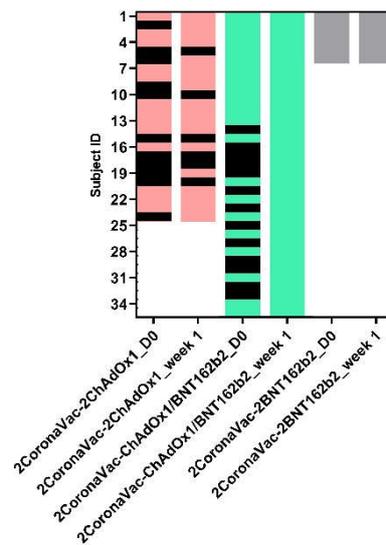
Fig. 3A Positive participant after first booster



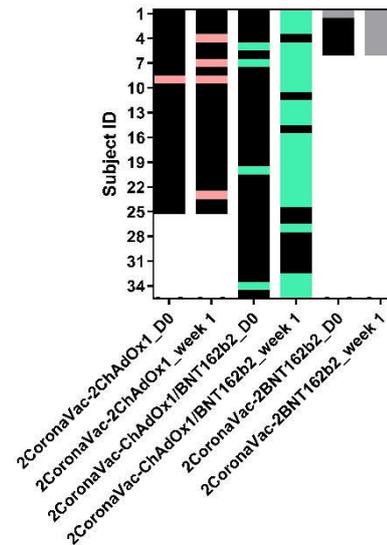
3B. Positive participant against Ancestral strain



3C. Positive participant against Delta varian



3D. Positive participant against Omicron variant



**Figure 3.** The proportion of participants with the level of microneutralization  $> 50\%$  against Ancestral, Delta and Omicron variant at day 0 (d0) and post first or second booster. A group of first booster recipients at d0.

#### 4. Discussion

This study was conducted between June 2021 and February 2022. CoronaVac vaccine has been used as the primary vaccination in many countries, including Thailand, but not exclusively. Other vaccination options in Thailand include homologous vaccination with ChAdOx1 or BNT162b2 (Pfizer-BioNTech) and heterologous prime boost by using CoronaVac-ChAdOx1. During the early phase of vaccine distribution in Thailand, there was the limited availability of mRNA vaccines (BNT162b2), with availability only beginning in June 2021. Healthcare workers and populations at risk for hospitalization and death from COVID-19 were prioritized early during vaccination. The first variant found in Thailand was characterized by the presence of the D614G mutation. The Alpha, Delta and Omicron VOCs emerged in Thailand in February, April 2021, and February 2022,

respectively. As vaccine-induced antibody responses wane overtime, vaccine boosters are thus recommended to maintain optimal protection against infection and severe COVID-19 disease [16]. Similarly, continued vaccine-induced immune responses are required for protection against immune evasion by emerging VOCs [17]. Participants in this study are health care workers who had received CoronaVac as the primary vaccination, followed by different booster regimens, as determined by the participant's own preference, workplace policy, availability of boosters and emergence of new variants of SARS-CoV-2. The primary vaccination program was implemented during the Alpha VOC predominance in Thailand. With the emergence of Delta VOC, a booster program was recommended by the Thai Ministry of Public Health and aimed to restore immunity against severe COVID-19 caused by Delta VOC. With the introduction of the Omicron VOC in February 2022, an additional, second, booster was recommended, especially for healthcare worker and individuals with high risk for severe disease.

From our findings, neutralizing titers 12 weeks after primary vaccination with the inactivated CoronaVac COVID-19 vaccine are low and inadequate to neutralize the ancestral strain of SARS-CoV-2 and the Delta and BA.1 Omicron VOCs. This is evident by the low rate of NT positivity and low mean inhibitory titers found in the serum of CoronaVac recipients at the time of their (first) ChAdOx1 booster (Figure 2A-2C). The rate of NT positivity and mean inhibitory titers increased rapidly by Day 7 after a single ChAdOx1 booster and remained so through Day 30. However, the levels of neutralization at Day 30 varied significantly, with moderate to low levels against the Delta and Omicron VOCs, with only a small proportion of participants developing neutralizing titers against Omicron at Day 7 and 30 after the ChAdOx1 booster. Although not directly measured from the 2CoronaVac-1ChAdOx1 group (the only group receiving a single ChAdOx1 booster dose), we can infer (from the 2CoronaVac-2ChAdOx1 and 2CoronaVac-ChAdOx1/BNT162b2 groups) that the single ChAdOx1 booster-derived neutralizing titers against SARS-CoV-2 ancestral strain remained unchanged for at least 160-180 days, as Day 0 blood sample in these two groups is collected 160-180 days post their first ChAdOx1 booster. The same is not the case for neutralizing titers against the Delta and Omicron VOCs, which seemed to have eroded further by 160-180 days after the ChAdOx1 booster. The 2CoronaVac-2BNT162b2 group, although small ( $n = 6$ ) allows to compare the humoral immunogenicity of the first BNT162b boost and compare it to the ChAdx1 booster, above. At 141 days post BNT162b boost (and at the time that the second BNT162b boost was applied), all subjects remained NT positive against the Delta VOC, in contrast to the first ChAdx1 boost received in the other groups. There were no differences in neutralization against ancestral strain and Omicron VOC.

In the groups that received a second boost dose, this was applied between 141 and 182 days after the first one. The effect of a second booster was rapid (measured at 7 days) and broad. Mean neutralizing against the ancestral strain did not change significantly, and this may have been caused by the already high and sustained titers generated by previous boosts. However, mean neutralization increased significantly against the Delta COV when a second ChAdOx1 ( $p = 0.0078$ ) or BNT162b2 ( $p < 0.001$ ) boost was used. Similarly, against the Omicron VOC, a second ChAdOx1 or BNT162b2 boost induced rapid neutralization. The data shows that a second BNT162b2 boost generated significantly ( $p = 0.034$ ) higher mean neutralizing than a second ChAdOx1 boost, suggesting that BNT162b2 might be more suitable for induction of antibody responses against Omicron VOC. Ultimately, in this study, the data show that the use of the BNT162b2 as both first and second booster generated the strongest responses against all strain and variants. An additional dose of BNT162b2 after 2 doses of CoronaVac as the primary vaccination showed a significant increase in the levels of neutralizing antibodies against Omicron in a study conducted in Brazil [18]. Our data support the use of BNT162b2 as a second booster might maintain adequate neutralization against Omicron.

In summary, these data indicate that people previously vaccinated with an inactivated CoronaVac SARS-CoV-2 vaccine can benefit from a single ChAdOx1 booster as early as one week after administration as this booster rapidly induced neutralizing

antibodies against both the ancestral strain, Delta VOC and, less so, against the Omicron VOC. The challenges caused by the arrival of new SARS-CoV-2 variants make two-dose booster regimen likely necessary to induce or maintain optimal levels of neutralizing antibodies, against these new variants, like Omicron VOC. The findings suggested that two doses of ChAdOx1 as the booster induced lowest activity against delta and omicron variants. A combination of a booster regimen by ChAdOx1 and BNT162b2 induced greater neutralization against Delta and Omicron than by two doses of ChAdOx1 but the levels of neutralization against Omicron were moderate. Two doses of BNT162b2 as the booster had the maximal activity against Omicron comparing to other booster regimens. The immune response against Omicron increased rapidly at 1 week after the fourth booster.

There are some limitations of this study, including the lack of a nonrandomized design and the limited number of participants. Further studies looking at the kinetics and durability of humoral immunity after single or repeated homologous and heterologous vaccine boosters will be required to document kinetics of humoral immune protection.

## 5. Conclusions

Vaccines to prevent SARS-CoV-2 infections are considered the most important approach for curbing the COVID-19 pandemic. Several COVID-19 vaccine platforms are available globally. Booster doses are necessary to enhance immunity against SARS-CoV-2 variants. Our study suggests that rapid and robust immunogenicity can be obtained with heterologous booster regimens against Omicron variant. A first dose of ChAdOx1 elicits at least some degree of neutralizing antibodies against Omicron VOC but these levels of neutralization decreased rapidly after 12 weeks post booster. Additional boosters are required. However, boosters, their type and frequency have to be carefully calibrated as neutralizing antibodies differ in their longevity and capacity to neutralize current and future circulating variants. Although limited by the number of subjects, our data suggest that using mRNA vaccines, at least as part of a booster regimen, provides higher neutralizing antibodies against new VOCs.

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**Institutional Review Board Statement:** The study was conducted in accordance with the Declaration of Helsinki and approved by the Research Ethics Review Committee, Faculty of Medicine, Chulalongkorn University (IRB No. 170.64).

**Informed Consent Statement:** Informed consent was obtained from all participants involved in the study.

**Data Availability Statement:** The supporting data for the findings of this study are available from the corresponding author upon reasonable request.

**Conflicts of Interest:** The authors declare no conflict of interest.

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