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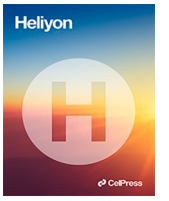

Immunogenicity and safety of heterologous versus homologous prime-boost schedules with inactivated and adenoviral vectored SARS-CoV-2 vaccines – A prospective multi-center study

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A B S T R A C T

Background: During the peak of Coronavirus disease (COVID-19) pandemic in Thailand when the emergence of delta variant reduced the efficacy of inactivated vaccine, Thailand had abundance of inactivated vaccine but mRNA vaccine was not available and the supply of adenoviral-vectored vaccine was limited. The heterologous vaccination using CoronaVac and ChAdOx1-nCoV-19 vaccines was applied. We aim to compare the immunogenicity of immune response of primary vaccination with homologous ChAdOx1 nCoV-19 and heterologous vaccination with CoronaVac and ChAdOx1 nCoV-19.

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Methods: A total of 430 adults, scheduled to receive ChAdOx1-nCoV-19 as their second dose of primary COVID-19 vaccination, were enrolled. Participants were classified into two groups based on the first dose vaccine as CoronaVac (heterologous group) or ChAdOx1 nCoV-19 (homologous group). The primary outcome was antibodies to the SARS-CoV-2 spike protein receptor binding domain (anti-RBD) titres at 28 days after the second dose of vaccination. Secondary outcomes were anti-RBD titres at 90 days, surrogate viral neutralizing test (sVNT) at 28 and 90 days, and adverse events.

Findings: In 358 participants with correct vaccine interval, the anti-RBD geometric mean titre ratio for the heterologous versus homologous group was 0.55 (95%CI; 0.44–0.067); $p < 0.001$ at day 28, and 0.80 (95%CI; 0.65–1.00); P = 0.05 at day 90. Median sVNT neutralizing activity was not significantly different in the heterologous versus homologous group at 28 days (93.5 vs 92.7 %); $p = 0.13$, but significantly higher in the heterologous group at day 90 (82.9 vs 76.4 %); $p = 0.01$.

Interpretation: The homologous vaccination resulted in higher anti-RBD titres at 28 days after vaccination, but titres in the homologous group showed more rapid decline at 90 days. In the sVNT assay, median neutralization was similar at 28 days, but was longer-lasting and higher in the heterologous group at 90 days.

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1. Research in context

1.1. Evidence before this study

We searched PubMed for research articles published from database inception until Mar 1, 2022, using the terms “SARS-CoV-2”, “COVID-19” “vaccine”, and “heterologous”. At the time of the search, there was one study involving heterologous vaccination by an inactivated vaccine and an adenoviral vectored vaccine. This study reported significantly higher SARS-CoV-2 RBD-specific antibody responses and neutralizing activities against both wild type and variants of concern with a heterologous vaccination regimen by an inactivated vaccine and an adenoviral vectored vaccine compared to homologous adenoviral vectored vaccination at one month. However, no data beyond one month was reported, indicating the need for further studies to evaluate the long-term efficacy and safety of this heterologous prime-boost COVID-19 vaccination approach.

1.2. Added value of this study

We demonstrated that the heterologous prime-boost COVID-19 vaccination regimen with inactivated vaccine and an adenoviral vectored vaccine achieved comparable immune response to the homologous adenoviral vectored regimen, with better humoral immune response than the reported homologous inactivated regimen. Furthermore, our results indicate that the immunity from the heterologous regimen lasted longer, as evidenced by higher surrogate neutralizing antibody levels at 90 days after complete vaccination compared to the homologous adenoviral vectored group.

1.3. Implications of all the available evidence

The heterologous prime-boost COVID-19 vaccination regimen with inactivated vaccine and an adenoviral vectored vaccine may offer advantages over both homologous vaccine regimens by achieving better immune response than the homologous inactivated vaccine regimen, while also exhibiting longer-lasting immunity than the homologous adenoviral vectored vaccine regimen, without any serious adverse events. These findings suggest that the heterologous prime-boost COVID-19 vaccination regimen may be a promising approach for enhancing vaccine efficacy and durability and warrant further investigation in larger clinical trials.

2. Introduction

In December 2019, Coronavirus disease 2019 (COVID-19) emerged in Wuhan, China, and progressed to a worldwide pandemic. By December 2021, ten vaccines including NVX-CoV2373 (Nuvaxovid [1], COVOVAX [2]), mRNA-1273 [3], BNT162b2 [4], 26 CE.COV2.S [5], ChAdOx1 nCoV-19 (Vaxzevria [6], Covishield [7]), BBV152 [8], BBIBP-CorV [9] and CoronaVac [10] had been approved by the World Health Organization (WHO) for emergency use. In Thailand, two vaccines became available in March 2021: CoronaVac for adults aged 18–59 years and ChAdOx1 nCoV-19 for elderly people $\geq$60 years. In July 2021 with the spread of the highly transmissible Delta variant (B. 1.617.2) which caused more severe disease and reduced vaccine-induced protection [11], Thailand had only a limited supply of ChAdOx1 nCoV-19 vaccine and no available mRNA vaccines, so heterologous vaccination with one dose of CoronaVac followed by a dose of ChAdOx1 nCoV-19 after a three-to-four-week interval for all age groups was approved by Ministry of Public Health. This regimen was hypothesized to produce a comparable immune response to the standard two doses of ChAdOx1 nCoV-19 with a shorter interval between doses, theoretically resulting in earlier protection. However, the immunogenicity and safety of this regimen has never been prospectively evaluated. Our objectives were to compare the immunogenicity and longevity of immune response following primary vaccination with homologous ChAdOx1 nCoV-19, or heterologous vaccination with CoronaVac and ChAdOx1 nCoV-19. We also sought to explore the safety of heterologous vaccination.

3. Methods

3.1. Study design and participants

This prospective cohort study sought to evaluate the immunogenicity and safety of homologous vaccination with ChAdOx1 nCoV-19 versus heterologous vaccination with CoronaVac and ChAdOx1 nCoV-19. A total of 430 participants scheduled to receive ChAdOx1 nCoV-19 as their second dose of primary vaccination regimen, were recruited from seven vaccination sites located in various regions of Thailand. These sites included Chulalongkorn University, MedPark hospital, Phramongkutklao hospital, Ramathibodi hospital, Royal Thai Air Force hospital (Sikan), and Siriraj hospital in Bangkok, Maharat Chiang Mai hospital and Maharat Nakhon Ratchasima hospital. The recruitment process was carried out in the interval between the first and the second dose of primary vaccination. The first dose of vaccination could be either CoronaVac or ChAdOx1-nCoV-19. The inclusion criteria were Thai nationals aged ≥18 years and the appropriate vaccine interval of each group. The exclusion criteria were previous COVID-19 infection, known or suspected allergy to the vaccine or its excipients, use of immunosuppressants, immunoglobulin or blood components within 3 months prior to vaccination, anatomical or functional asplenia, drug abuse, alcoholism, autoimmune diseases, or immunodeficiency, pregnancy, and prior use of biological agents. All participants received the second dose of vaccination with ChAdOx1 nCoV-19 using vaccine distributed by the Thai government. The heterologous group were vaccinated with CoronaVac followed by one dose of ChAdOx1 nCoV-19 after three-to-four-weeks; the homologous group received a second dose of ChAdOx1 nCoV-19 eight to sixteen weeks after the first dose [6]. The study protocols were approved by the Central Research Ethics Committee of Thailand (COA-CREC072/2021). Written informed consent was obtained from all participants prior to enrollment. The authors vouched for the accuracy and completeness of the data and for the fidelity of the study to the protocol. This trial was registered on Thai Registry Trial Registry (TCTR20210913001) and was solely sponsored by the Royal College of Physicians of Thailand.

3.2. Vaccination

3.2.1. CoronaVac

CoronaVac is an inactivated whole-virion vaccine with aluminum hydroxide as an adjuvant, developed by Sinovac Life Sciences, Beijing, China. The vaccine was administered intramuscularly into the deltoid region. It was approved for adults aged 18–59 years with a two-dose schedule administered 21–28 days apart [10].

3.2.2. ChAdOx1 nCoV-19

ChAdOx1 nCoV-19 is a viral-vectored SARS-CoV-2 vaccine, developed by University of Oxford and AstraZeneca. The vaccine was administered intramuscularly into the deltoid region. It was approved for adults aged above 18 years with two-dose schedule of eight

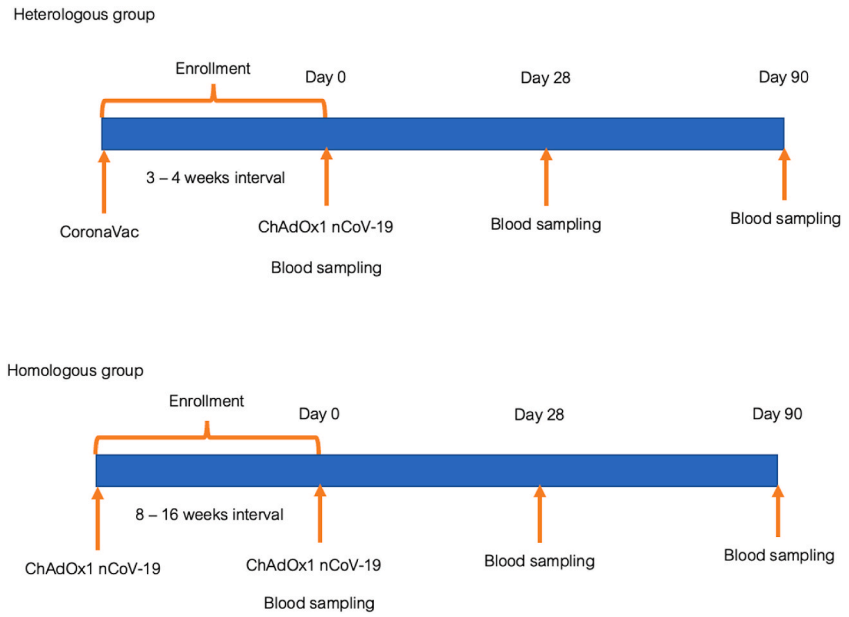

Fig. 1. Study schedule
The schedule of enrollment and blood sampling for anti-RBD and sVNT was shown. In heterologous group, the enrollment process was performed during the interval between the first dose, CoronaVac, and the second dose, ChAdOx1 nCoV-19. In homologous group, the enrolment occurred during the interval between the first and the second dose of ChAdOx1 nCoV-19.
anti-RBD; anti-retinol binding globulin; sVNT: surrogate viral neutralizing test.

to sixteen weeks apart [6].

3.3. Immunological assessment

Three serum samples were obtained: pre-vaccination on the day that the participants received the second dose, at 28 days and 90 days after receiving the second dose. All blood samples were analyzed at the Division of Virology, Department of Microbiology, Faculty of Medicine, Chulalongkorn university. Quantitative determination of antibodies to the SARS-CoV-2 spike protein receptor binding domain (anti-RBD) and surrogate viral neutralizing test (sVNT) to original strain were performed, using Elecsys® Anti-SARS-CoV-2 S (Roche Diagnostics, Basel, Switzerland) and GenScript cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit, respectively (see Fig. 1).

3.4. Safety assessment

Local and systemic solicited adverse reaction including pain, tenderness, erythema, swelling, headache, fatigue, myalgia, fever, chills, arthralgia, vomiting, diarrhea, and use of antipyretic, or pain medication were recorded daily in the first seven days after vaccination via online questionnaire, instant messaging application and telephone call by research staff.

Unsolicited adverse events were collected through day 90 by spontaneous reporting from participants and by research staff at visits on day 28 and day 90.

3.5. Outcomes

3.5.1. Primary outcome

The primary immunological outcome was anti-RBD titres at 28 days after the second dose of the heterologous or homologous vaccination.

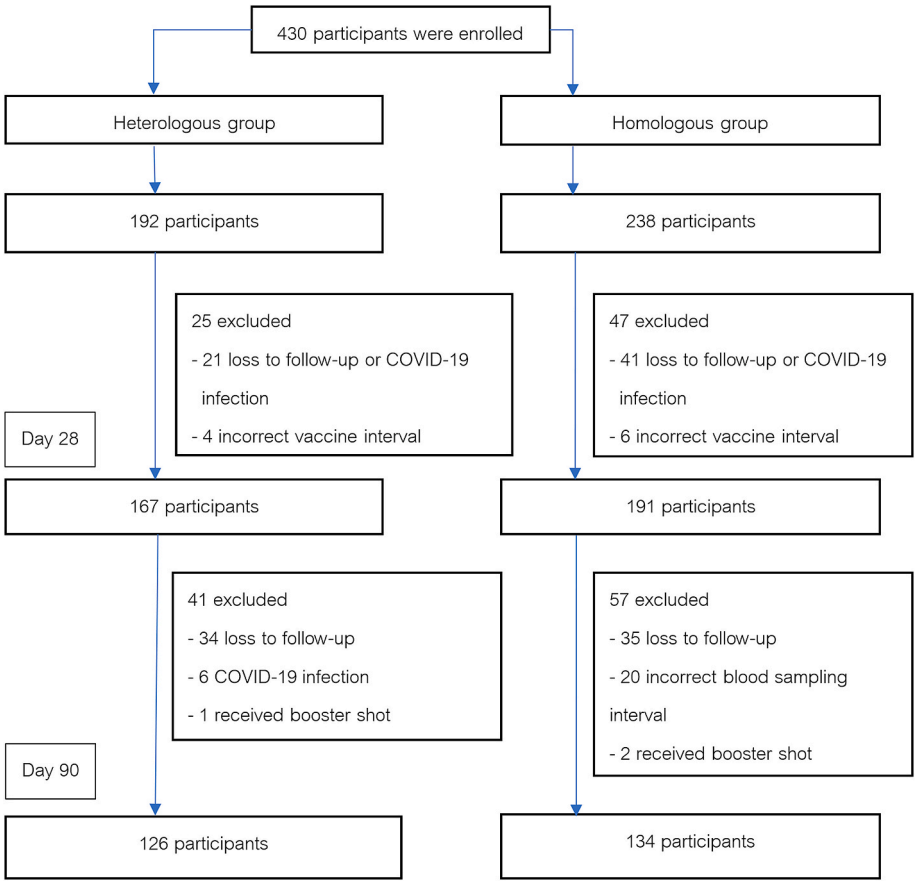

Fig. 2. Study flow diagram.
430 participants were enrolled and assigned according to their vaccination regimen.

3.5.2. Secondary outcome

The secondary immunological outcomes included sVNT at day 28 and day 90 and anti-RBD at day 90 after the second dose of vaccination. Safety outcomes were local and systemic solicited adverse reaction in the first seven days after vaccination and unsolicited adverse events in 90 days after vaccination.

3.6. Statistical analysis

The sample size was calculated based on geometric mean (GMT) and geometric standard deviation (GSD) of anti-RBD titers after 2 doses of ChAdOx1 nCoV-19 vaccination [12]. If the heterologous vaccine resulted in a 25 % reduction in GMTs and GSD, enrolling 150 participants per group would give 80 % power to detect a magnitude of this difference at a 2-sided significance level of 5 %. Baseline demographics and characteristics, including age, gender and comorbidities were reported using descriptive statistics: categorical data as frequency (percentage) and continuous data as mean (standard deviation), or median (interquartile range) as appropriate. Formal comparisons of continuous characteristics between study groups were made with a Wilcoxon rank test, and categorical characteristics were compared with a Fisher's exact test.

Immunogenicity analysis including anti-RBD titer and surrogate viral neutralizing test were performed at day 28 and 90. Formal comparisons of the GMTs by study group were made using a regression model with log transformed titres as the outcomes. Model estimates were back transformed to give GMT ratios (GMTR) and 95%CI.

The safety data were descriptively analyzed in all participants. Frequencies and percentages of subjects experiencing each solicited adverse events were presented for each severity of the symptom. Formal comparisons between study groups were made with a Fisher's exact test. All reported unsolicited adverse events with onset occurring during the 28 days after vaccine administration were assessed for severity and as either related or not related to vaccine by the investigators.

4. Results

4.1. Enrollment

Between August and November 2021, a total of 430 participants who received ChAdOx1 nCoV-19 as the second dose of vaccination regimen were enrolled. One hundred nighty-two participants receiving CoronaVac and 238 participants receiving ChAdOx1 nCoV-19 as their first dose of primary vaccination regimen were included in the heterologous and homologous group respectively. Twenty-five participants in the heterologous group and 47 in the homologous group were then excluded from the immunogenicity analysis due to loss to follow-up, COVID-19, or incorrect vaccine interval. At 28 days, 358 participants were available for immunogenicity analysis, comprising of 167 participants in the heterologous group and 191 participants in the homologous group. Fig. 2 showed the study flow diagram.

4.2. Demographic data

The median (IQR) age of participants was 43 (IQR 20–56) years in the heterologous group and 42 (IQR 30–55) years in the

Table 1
Baseline characteristics.

	Total	Heterologous group	Homologous group	*p*-value[a]
	N = 368	**N = 171**	**N = 197**	
Age, years **(median, IQR)**	43 (28–55)	43.0 (20–56)	42.0 (30–55)	0.12
Age group**, n (%)**				0.88
< 50	235 (64 %)	109 (64 %)	126 (64 %)	
50–59	61 (17 %)	27 (16 %)	34 (17 %)	
>= **0**	72 (20 %)	35 (20 %)	37 (19 %)	
Sex, n (%)				0.40
Female	194 (53 %)	86 (50 %)	108 (55 %)	
Male	174 (47 %)	85 (50 %)	89 (45 %)	
Comorbidities**, n (%)**				
Any	19 (5 %)	3 (2 %)	16 (8 %)	0.008
Diabetes	16 (4 %)	2 (1 %)	14 (7 %)	0.008
Cardiovascular	6 (2 %)	1 (1 %)	5 (3 %)	0.22
Respiratory	2 (1 %)	1 (1 %)	1 (1 %)	1.0
Renal	1 (0 %)	0 (0 %)	1 (1 %)	1.0
Time between dose 1 and 2 (days) **(median, IQR)**	56 (27–84)	27 (24–28)	83 (76–84)	<0.001
Time between dose 2 and d28 blood collection (days) **(median, IQR)**	28 (28–28)	28 (24–32)	28 (28–28)	0.85

[a] Formal comparisons of continuous characteristics between groups made with a Wilcoxon rank sum test; categorical characteristics were compared with a Fisher's exact test.

homologous group. Fifty-three percent of participants were female. Nineteen participants had one or more comorbidities. Most were type 2 diabetes mellitus and were in the homologous group. Time between the 1st and the 2nd dose were 24–28 days in the heterologous group and 76–84 days in the homologous group. Table 1 showed the demographic data of participants.

4.3. Immunogenicity analysis

4.3.1. Anti-RBD titers

At 28 days after the second dose of vaccination, in the heterologous group, 21 participants were excluded due to loss to follow-up or COVID-19 infection and 4 participants were excluded due to incorrect vaccine interval. In the homologous group, 41 participants were excluded due to loss to follow-up or COVID-19 infection and 6 participants were excluded due to incorrect vaccine interval. One Sixty-seven and 191 participants in the heterologous and the homologous group were included respectively.

The GMTs of Anti-RBD before the second dose were 3.00 U/mL (95%CI; 2.38–3.78) in the heterologous group and 62.54 U/mL (95%CI; 51.84–75.46) in the homologous group. At 28 days after the second dose, the GMTs increased to 643.22 U/mL (95 % CI; 540.15–765.97) in the heterologous group and 1179.15 U/mL (95%CI; 1042.88–1333.23) in the homologous group. The GMTR of the heterologous to the homologous group was 0.55 (95%CI; 0.44–0.67).

At 90 days after the second dose, 41 participants in the heterologous group and 57 participants in homologous group were excluded. One hundred twenty-six participants in the heterologous group and 134 participants the homologous group were included at this time point. The GMTs of the heterologous and the homologous group were 464.83 U/mL (95 %; 396.39–545.09) and 578.30 U/mL (95%CI; 497.62–673.07), respectively. The GMTR at 90 days was 0.80 (95%CI; 0.65–1.00). Table 2, Figs. 3 and 4A showed the results of anti-RBD.

4.3.2. Surrogate viral neutralizing test (sVNT)

Surrogate viral neutralizing tests were performed in participants with available blood samples at all three time points. One hundred twenty-eight participants in the heterologous group and 153 participants in the homologous group were included in this analysis. Before the second dose of vaccination, the neutralizing activities were 15.0% inhibition (IQR; 15.0–15.0) in the heterologous group and 41.8% inhibition (IQR; 15.0–58.8) in the homologous group. The result of the homologous group was significantly higher than that of the heterologous group (p-value <0.001).

At 28 days after the second dose, the neutralizing activities were not different between the heterologous and the homologous group, [93.45 (IQR; 88.7–95.6) vs. 92.7 (IQR; 84.5–95.5), p-value = 0.099]. At 90 days, in contrast with the result at day 28, the neutralizing activities of the heterologous group were significantly higher than that of homologous group [82.9 (IQR; 70.6–91.1) vs. 76.7 (IQR; 60.3–89.5) percent inhibition, p-value = 0.014]. Table 3 and Fig. 4C showed the results of sVNT.

4.3.3. Safety

A total of 430 participants, 192 in the heterologous group and 238 in the homologous group, were included in the safety analysis. Within seven days after the second dose, solicited local adverse effects occurred in 52.4 % in the heterologous group and 43.6 % in the homologous group. The most common injection site reactions were pain, swelling and redness with most events being mild and moderate in severity. Swelling at injection were reported more frequently in the heterologous group than in the homologous group (18.4 % vs.4.7 %) (Fig. 5). The incidence of solicited systemic adverse events after the second dose were reported similarly in both heterologous and homologous group (50.8 % vs.48.5 %) The most common systemic adverse effects were feverish, myalgia, and malaise with most events being mild and moderate in severity. Chills and loose stool were reported more frequently in the heterologous group than in the homologous group (11.0 % vs. 2.9 %) and (13.6 vs. 6.0 %) respectively. The occurrence of higher grade of feverish feeling (moderate-severe) were reported more frequently among heterologous participants than homologous participants (9.9 % vs.

Table 2
Anti-RBD geometric mean titres ratios of heterologous to homologous group.

Group	Heterologous group	Homologous group	GMTR (95%CI)
Immunogenicity analysis	N = 167	N = 191	
Before 2nd dose	3.00 (2.38–3.78)	62.54 (51.84–75.46)	
28 days after 2nd dose	643.23 (540.15–765.97)	1179.15 (1042.88–1333.23)	0.55 (0.44–0.67) ($p < 0.001$)
90 days after 2nd dose	(N = 126) 464.83 (396.39–545.09)	(N = 134) 578.30 (497.62–673.07)	0.80 (0.65–1.0) ($p = 0.05$)
Post-hoc analysis including all available data	**N = 171**	**N = 197**	
Before 2nd dose	2.96 (2.35–3.72)	62.34 (51.83–74.99)	
28 days after 2nd dose	645.58 (543.22–767.21)	1159.27 (1024.94–1311.20)	0.56 (0.45–0.69) (p^a <0.001)
90 days after 2nd dose	(N = 127) 459.38 (391.57–538.93)	(N = 135) 575.48 (495.59–668.25)	0.80 (0.64–0.99) ($p^a = 0.04$)

Data shown are geometric mean titer (95%CI); RBD: receptor-binding domain; GMTR: Geometric mean titre ratio.
[a] p-values are from linear regression models used to calculate the GMTRs.

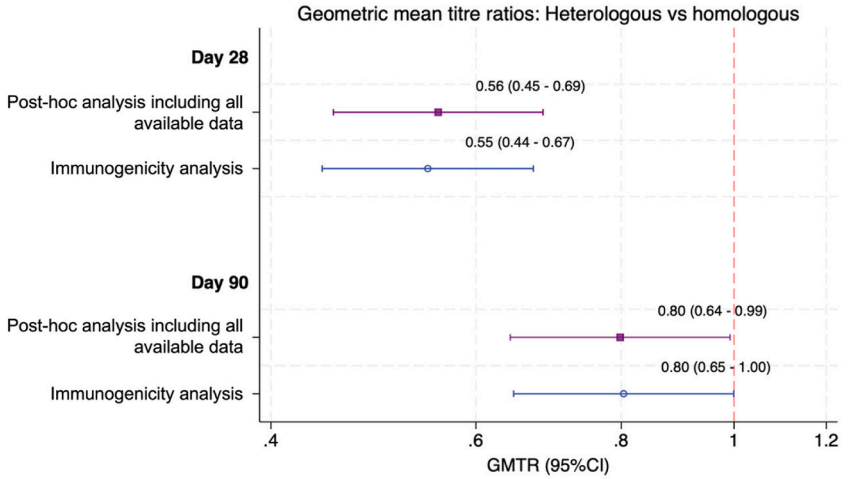

Fig. 3. Geometric mean titre ratios of anti-RBD at 28 and 90 days after completing the primary vaccination schedule
Lines indicate geometric mean titre ratios and 95 % confidence intervals
GMTR: geometric mean titre ratio.

2.5 %, $p = 0.02$).

No serious adverse events and deaths were reported throughout three month of study period in both homogeneous and heterogeneous prime boost regimen. Table 5 showed incidence and severity of each adverse events.

4.4. Post-hoc analysis

4.4.1. Analysis with all available data

We performed the analysis with all available blood samples including participants with incorrect blood sampling intervals.

4.4.2. Anti-RBD titers

At 28 days after the second dose, 171 and 197 participants in the heterologous and the homologous group were included respectively. The GMTs before the second dose were 2.96 U/mL (95%CI; 2.35–3.72) in the heterologous group and 62.34 U/mL (95% CI; 51.83–74.99) in the homologous group. At 28 days after the second dose, the GMTs increased to 645.58 U/mL (95 % CI; 543.22–767.21) and 1159.27 U/mL (95%CI; 1024.94–1311.20), respectively. The GMTR was 0.56 (95%CI; 0.45–0.69). At 90 days after the second dose, 127 and 135 participants in the heterologous and the homologous group were available for analysis. The GMTs were 459.38 U/mL (95 %; 391.57–538.93) and 575.48 U/mL (95%CI; 495.59–669.25), respectively. The GMTR at was 0.80 (95%CI; 0.64–0.99). Fig. 4B showed the showed the results of anti-RBD in this analysis.

4.4.3. Surrogate viral neutralizing test (sVNT)

One hundred twenty-nine participants in the heterologous group and 157 participants in the homologous group were included in this analysis. Before the second dose, the neutralizing activities were 15.0% inhibition (IQR; 15.0–15.0) in the heterologous group and 42.0% inhibition (IQR; 15.0–59.4) in the homologous group. The result of the homologous group was significantly higher than that of the heterologous group (p-value <0.001).

At 28 days after the second dose, the neutralizing activities were not different between the heterologous and the homologous group, [93.4 (IQR; 88.9–95.6) vs. 92.3 (IQR; 84.3–95.5), p-value $= 0.10$]. At 90 days, the neutralizing activities of the heterologous group were significantly higher than that of homologous group [82.9 (IQR; 70.5–91.0) vs. 76.0 (IQR; 60.2–89.9) percent inhibition, p-value $= 0.01$]. Fig. 4D showed the showed the results of sVNT in this analysis.

The results of these analysis were not different from the immunogenicity analysis.

4.5. Subgroup analysis

We performed a post-hoc subgroup analysis of anti-RBD at 28 days after the second dose of vaccination by stratification according to age and sex. The age was stratified into two subgroups, <60 and ≥ 60. The GMTs of the homologous group were higher than those of heterologous group in both subgroups. The GMTs were significantly lower in participants aged above 60 years old than those age lower than 60 in both regimens. When stratified by sex, the GMTs of the homologous group were higher than those of the heterologous group in both male and female subgroups. Table 4 showed the results of subgroup analysis.

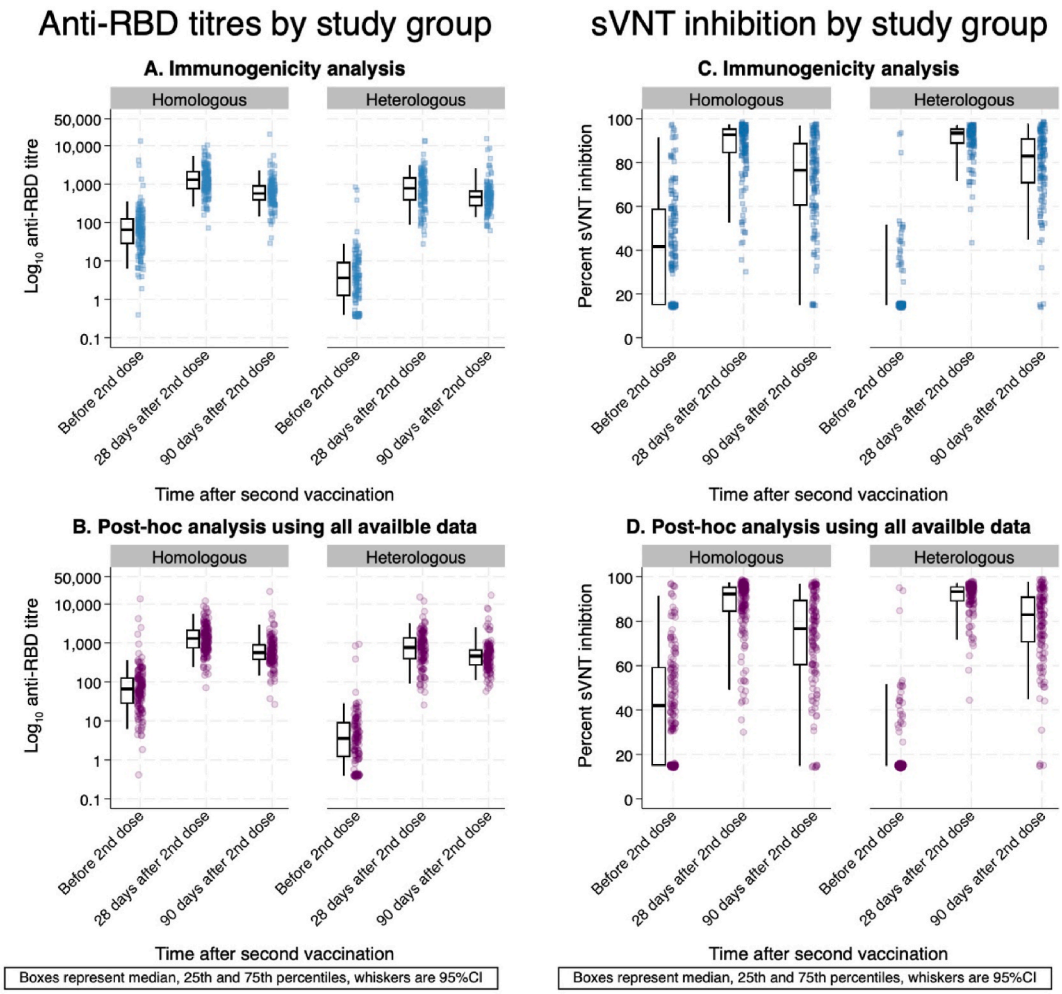

Fig. 4. Anti-RBD titres by timing relative to the second dose vaccination in A) the immunogenicity population and B) post-hoc analysis using all available data (left hand panel), and percent inhibition by surrogate viral neutralizing test in C) the immunogenicity population and D) post-hoc analysis using all available data (right hand panel).
Boxes represent median, 25th and 75th percentiles. Whiskers represent 95 % confidence interval.
Anti-RBD: anti-retinol binding domain; sVNT: surrogate viral neutralizing test.

Table 3
Median (Q1, Q3) percent inhibition by surrogate viral neutralizing test.

Group	Heterologous group	Homologous group	*p*-value[a]
Immunogenicity analysis	N = 128	N = 153	
Before 2nd dose	15.0 (15.0–15.0)	41.6 (15.0–58.9)	<0.001
28 days after 2nd dose	93.5 (88.7–95.6)	92.7 (84.3–95.5)	0.13
90 days after 2nd dose	(N = 126)	(N = 134)	0.01
	82.9 (70.6–91.0)	76.4 (60.3–88.9)	
Post-hoc analysis including all available data	**N = 129**	**N = 157**	
Before 2nd dose	15.0 (15.0–15.0)	42.0 (15.0–59.4)	<0.001
28 days after 2nd dose	93.4 (88.9–95.6)	92.3 (84.3–95.5)	0.10
90 days after 2nd dose	(N = 127)	(N = 135)	0.01
	82.9 (70.5–91.0)	76.1 (60.2–89.9)	

[a] *p*-values derived from Wilcoxon rank-sum tests.

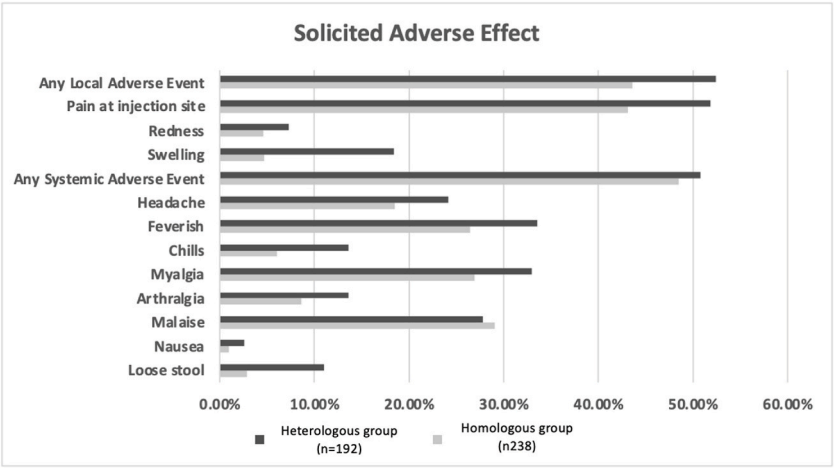

Fig. 5. Solicited local and systemic adverse effects within 7 days after the second dose of vaccine.

Table 4
Subgroup analysis by age and sex of anti-RBD at 28 days after the second dose.

	Heterologous group	Homologous group	GMTR (95%CI)	*p* for interaction[a]
Age				0.07
<60 years				
Number of participants	133	155		
Anti-RBD	729.82 (604.66–880.88)	1209.04 (1064.80–1372.81)	0.60 (0.48–0.75)	
>/ = 60 years				
Number of participants	34	36		
Anti-RBD	392.36 (257.99–597.02)	1058.67 (732.57–1529.93)	0.37 (0.21–0.64)	
Sex				0.29
Male				
Number of participants	83	87		
Anti-RBD	692.10 (535.19–895.02)	1129.05 (960.31–1327.44)	0.61 (0.45–0.83)	
Female				
Number of participants	84	104		
Anti-RBD	598.32 (470.20–761.35)	1222.76 (1018.73–1467.66)	0.49 (0.36–0.66)	

Data shown are geometric mean titers (95%CI); RBD: receptor-binding domain; GMTR: Geometric mean titre ratio.
[a] *p* for interaction calculated from a regression model with an interaction term between treatment group and each subgroup.

Table 5
Adverse events.

	Heterologous group (n = 192)	Homologous group (n = 238)	*p*-value[a]
Local side effect	100 (52.4 %)	103 (43.6 %)	0.08
Pain at injection site	99 (51.8 %)	101 (43.16 %)	0.07
Redness	14 (7.3 %)	11 (4.62 %)	0.30
Swelling	35 (18.4 %)	11 (4.7 %)	<0.001
Systemic side effect	97 (50.8 %)	114 (48.5 %)	0.63
Headache	46 (24.1 %)	43 (18.5 %)	0.15
Feverish	64 (33.5 %)	62 (26.4 %)	0.11
Chills	26 (13.6 %)	14 (6.0 %)	0.007
Myalgia	63 (33.0 %)	53 (26.9 %)	0.02
Arthralgia	26 (13.6 %)	20 (8.6 %)	0.12
Malaise	53 (27.8 %)	65 (29.0 %)	1.0
Nausea	5 (2.6 %)	2 (0.9 %)	0.25
Loose stool	21 (11.0 %)	10 (2.9 %)	0.008

[a] Formal comparisons between study groups were made with Fisher's exact test.

5. Discussion

Heterogenous vaccination had been implemented in several countries due to limited vaccine supplies at the peak of the COVID-19 pandemic. The effectiveness of the combination of different types of vaccines was evaluated. The heterologous vaccination with an

ChAdOx1 nCoV-19 followed by a mRNA BNT162b2 induced higher antibody and T cell responses to SARS-CoV-2 than the homologous ChAdOx1 nCoV-19 vaccination and comparable to the homologous BNT162b2 [13–17]. Heterologous ChAdOx1 nCoV-19/mRNA-1273 vaccination also showed similar results [18,19]. However, heterologous BNT162b2/ChAdOx1 nCoV-19 vaccination did not meet the non-inferiority criteria to homologous BNT162b2 vaccination [17].

Studies regarding heterologous inactivated and adenoviral vector COVID-19 vaccine regimen have been reported [20–22]. Two studies conducted by Wanlapakorn N et al. showed that at approximately one month after completion of the heterologous regimen, the GMT of total RBD immunoglobulin were similar between the heterologous group and the homologous group. Neutralizing activities specific to wild-type SARS-CoV-2 and alpha, beta, and delta variants of concern were high in both groups [20,21]. One study further explored cell-mediated immune response and found that the heterologous group also showed strong subtracted IFN-γ response [20]. Another preliminary study at the early Delta variants circulation showed similar result [22].

In our study with relatively older population, the total anti-RBD at 28 days after complete vaccination in the heterologous group were lower than that of the homologous group. The surrogate neutralizing antibodies were both equally high. Since neutralizing antibody levels were more predictive of immune protection from symptomatic COVID-19 infection, the lower level of total anti-RBD in the heterologous group may not demonstrate lower immune protection. The difference of anti-RBD level prior to the 2nd dose could be attributed to the difference in immunogenicity between CoronaVac and ChAdOx1 nCoV-19. Given our approach of analyzing both heterologous and homologous regimens as complete vaccination regimens, it wound not be necessary to adjust the anti-RBD or surrogate neutralizing antibodies at 28 days for the baseline differences between the interval of the first and the second dose.

At 90 days after complete vaccination, the anti-RBD waned in both groups but more rapidly in the homologous group. The surrogate neutralizing antibodies in the heterologous group showed higher percent inhibition than the homologous group. These results may imply that the immunity from heterologous regimen was not different from homologous regimen for viral inhibition at 28 days after complete vaccination and lasted longer through 90 days after vaccination.

The findings of our study indicated that the heterologous prime-boost vaccination regimen with CoronaVac and ChAdOx1 nCoV-19 achieved equivalent immune response to homologous ChAdOx1 nCoV-19 regimen but with a shorter vaccine interval and resulted in higher immune response than homologous CoronaVac regimen [23]. However, our results also showed that the heterologous CoronaVac/ChAdOx1 nCoV-19 regimen had limited immune protection during the four weeks after the first dose, which may be a concern for population at high risk of infection.

In randomized, controlled, phase 2/3 trial of homologous ChAdOx1 nCoV-19 regimen, there were no differences of anti-spike IgG responses and live SARS-CoV-2 microneutralization assay across age groups up to above 70-year [24]. The regimen also showed similar vaccine efficacy in both younger (18- to 64-year-old) and older (above-65-year-old) age group [6]. On the other hand, some studies showed that homologous CoronaVac regimen produced lower antibody response in elderly compared with younger age group [25–27]. In the subgroup analysis of our study, anti-RBD of homologous ChAdOx1 nCoV-19 group were significantly higher than heterologous CoronaVac/ChAdOx1 nCoV-19 group across all age groups. Participants aged over 60 had significantly lower anti-spike antibodies of SARS-CoV-2 than those with lower age in both sub-groups. These results may reflect the lower immunogenicity in the elderly of CoronaVac.

In heterologous inactivated and adenoviral vector COVID-19 vaccine regimen study by Wanlapakron N et al., univariate and multivariate analysis of variables including age showed no effect on the immunogenicity of different vaccine regimens [20]. However, the maximum age in the heterologous group was 64 year and may not represent immunogenicity of the regimen in older population. In our study, twenty percent of participants were older than 60-year with the oldest up to 75-year-old in the heterologous group and 84-year-old in the homologous group. The higher proportion of the elderly and the higher age in our study may explain the difference of the results.

We did not conduct a comparative analysis between the heterologous CoronaVac/ChAdOx1 nCoV-19 regimen and the homologous CoronaVac regimen, as our study did not focus on the use of a homologous CoronaVac regimen, which was already discouraged during the period of our research.

The overall incidence of local and systemic reactogenicity after the second dose of vaccination were comparable in both vaccine regimens and were comparable in that reported in a phase 3 clinical trial of ChAdOx1 nCoV-19 [6]. Most adverse events were mild. However, the incidence of swelling at injection site, chills, and having loose stool were reported more frequently in the heterologous group. This might be because the participants in the heterologous group have not been exposed to adenoviral-vectored vaccine and might have more reaction than those with previous exposure in the homologous group. In our study, a small number of participants did not rate the severity of the adverse events. We assumed that those unreported severity were not severe because none of the participants needed to seek medical attention and no serious or fatal adverse events were reported in both groups throughout 3-month of follow up period.

Our study had several strengths. Firstly, it provides valuable information regarding the immunogenicity of heterologous prime-boost COVID-19 vaccination with CoronaVac and ChAdOx1 nCoV-19, which is a pertinent topic in light of the limited vaccine supplies during the COVID-19 pandemic. Secondly, it included a large number of participants, including those over 60 years of age, providing valuable insights into the immunogenicity of the vaccine regimens in older population. Thirdly, we followed up with participants for 90 days after complete vaccination, providing information on the durability of immune responses.

However, our study had limitations. Firstly, observational studies are subject confounding. We conducted analyses stratified by age group and sex, and the prevalence of comorbidities was low in the cohort, but the possibility of other unobserved confounding cannot be discounted. Secondly, we did not assess neutralizing antibodies against other variants of concern, and the protection conferred by the original SARS-CoV-2 strain might not be effective against the Delta and Omicron variants that emerged. Thirdly, our study did not include an assessment of T cell responses, which are important components of the immune response to SARS-CoV-2. However, the

study by Wanlapakron N et al. demonstrated an increase in interferon-gamma responses after ex vivo cell stimulation with SARS-CoV-2 antigens following heterologous CoronaVac/ChAdOx1 nCoV-19 vaccination [20], suggesting that this regimen also induced T cell responses. Future studies could provide a more comprehensive understanding of the cell-mediated immune response to heterologous prime-boost COVID-19 vaccination with CoronaVac and ChAdOx1 nCoV-19. Fourthly, the vaccine lots used for each group were not controlled, and this variation in vaccine lots may result in differences in vaccine immunogenicity. Fifthly, we did not assess baseline anti-RBD level prior to the first vaccination. While our study exclusively enrolled individuals without a documented history of prior SARS-CoV-2 infection, it is important to acknowledge that some participants may have experienced asymptomatic infection, which could effect their post-vaccination anti-RBD antibody levels. These limitations may impact the generalizability of our study.

Although our results may not be applicable for the current situation where sub-variants of Omicron are dominating in which the two regimens may not protect against infection well enough and the popularity and availability of these two vaccines are shrunken, it provided knowledge as the initiative about the potential benefit of using heterologous vaccination started by the inactivated whole-virion vaccine and followed by viral-vectored vaccine. The prime-boost vaccination strategy, as demonstrated by our study, has the potential to be a valuable tool in controlling future pandemics, particularly in situations where there are limited vaccine supplies or when the available vaccines are not highly effective. Furthermore, future studies could explore the potential of heterologous prime-boost vaccination as a strategy for enhancing immune responses in the development of vaccines for other infectious diseases.

Data availability statement

Data associated with the study has not been deposited into a publicly available repository and data will be made available on request.

CRediT authorship contribution statement

Pawat Phuensan: Writing - review & editing, Writing - original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Jarongkorn Sirimongkolkasem:** Writing - review & editing, Writing - original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Terapong Tantawichien:** Writing - review & editing, Validation, Supervision, Conceptualization. **Jeerath Phannajit:** Methodology, Formal analysis. **Stephen J. Kerr:** Writing - review & editing, Software, Formal analysis. **Pokrath Hansasuta:** Resources, Investigation. **Prawat Chantharit:** Investigation. **Adisorn Wongsa:** Investigation. **Pusit Fuengfoo:** Investigation. **Anutra Chittinandana:** Supervision, Investigation. **Kriengsak Vareesangthip:** Investigation. **Methee Chayakulkeeree:** Writing - review & editing, Investigation. **Sureeporn Jangsirikul:** Investigation. **Araya Schmidt:** Investigation. **Kanyika Wanvimonsuk:** Investigation. **Poramed Winichakoon:** Investigation. **Rattagan Kajeekul:** Investigation. **Wichai Prayoonwiwat:** Supervision, Investigation. **Rungsun Rerknimitr:** Writing - review & editing, Writing - original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Rungsun Rerknimitr reports financial support was provided by The Royal College of Physicians of Thailand. If there are other authors, they 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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