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Immunogenicity of heterologous inactivated and adenoviral-vectored COVID-19 vaccine: Real-world data



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ABSTRACT

Limited data are available on the responses to heterologous vaccine regimens for SARS-CoV-2, especially among countries using inactivated and adenoviral-vectored vaccines. A total of 77 participants who received heterologous inactivated COVID-19 vaccine (CoronaVac) and adenoviral-vectored vaccine (AZD1222) were enrolled in our study. There were two comparison groups vaccinated with the homologous CoronaVac (N = 79) and AZD1222 (N = 78) regimen. All sera samples were tested for anti-receptor-binding-domain IgG (anti-RBD IgG) using a chemiluminescent microparticle immunoassay (CMIA). The neutralizing activity in a subset of serum samples was tested against the original Wuhan strain and variants of concern, B.1.1.7, B.1.617.2 and B.1.351, using an enzyme-linked immunosorbent assay (ELISA)-based surrogate virus neutralization test (sVNT). The heterologous CoronaVac/AZD1222 vaccine induced higher levels of anti-RBD IgG than that of two-dose homologous CoronaVac or AZD1222 vaccines ($p < 0.001$). Sera samples of the CoronaVac/AZD1222 vaccine recipients elicited higher neutralizing antibody activity against the original Wuhan and all variants of concern than in the recipients of the two-dose CoronaVac. The heterologous CoronaVac followed by AZD1222 is an alternative regimen to combat with the SARS-CoV-2 variants in case of vaccine shortage with improved immunogenicity compared to the homologous CoronaVac regimen.

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1. Introduction

Limited data are available on the responses to heterologous vaccine regimens for SARS-CoV-2, especially among countries using inactivated and adenoviral-vectored vaccines. In the Com-COV trial, heterologous prime-booster combinations of the adenoviral-vectored (AZD1222) and BNT162b2 mRNA vaccines induced higher serum anti-spike IgG and neutralizing antibody titers than the homologous efficacy-proven adenoviral-vectored two-dose regimen [1]. The CombiVacS trial found that the Pfizer's BNT162b2 mRNA vaccine given as a second dose in individuals prime vaccinated with AZD1222 induced a robust immune response, with an acceptable and manageable reactogenicity profile [2]. Nevertheless, the Com-COV trial found an increase in systemic reactogenic-

ity after booster vaccination reported by participants in heterologous AZD1222-BNT162b2 schedules in comparison to homologous vaccine schedules [3].

Some countries have recommended the heterologous vaccine regimen, offering the mRNA vaccines as a second dose to young people who have already received the AZD1222 vaccine because of concerns about vaccine-induced thrombotic thrombocytopenia (VITT) [4]. A prospective observational cohort study in Germany showed that the heterologous vaccine regimen (AZD1222/BNT162b2) at a 10–12 week vaccine interval induced similar anti-RBD IgG responses but increased T-cell responses and neutralization capacity against SARS-CoV-2 alpha and beta variant compared with homologous AZD1222 and BNT162b2 vaccination [5]. Further research is required to comprehensively elucidate the immunological implications following different vaccine types and administration schedules.

Thailand has imported CoronaVac, which was developed by Sinovac Life Sciences, Beijing, China, since February 2021. Several phase 3 studies have shown acceptable safety and efficacy against

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symptomatic COVID-19 following two-dose CoronaVac vaccination [6–7]. Health professionals were the first prioritized group receiving two-dose CoronaVac in Thailand at a 3–4-week interval. Adenoviral-vectored vaccine, imported from Korea in March 2021 and later produced from the Siam Bioscience company (Nonthaburi, Thailand) was initially prioritized for the elderly above 60 years of age. The adenoviral-vectored vaccine (AZD1222) was administered two doses at 10 weeks apart. Preliminary studies have found the two-dose CoronaVac regimen induced a lower, but acceptable, immune response compared to the two-dose AZD1222 regimen with a shorter interval between two doses [8]. A 10-week interval for AZD1222 vaccine was established based on recommendations of the Thailand FDA and efficacy studies identifying that a waiting period of <6 weeks resulted in lower immune stimulation than a period of 10 weeks [9]. Thailand started vaccinations with the inactivated CoronaVac on 28 February 2021, and AZD1222 vaccine on 16 March 2021. The CoronaVac vaccine was associated with a rare focal neurological syndrome characterized by numbness, or sometimes weakness, in the limbs [10]. Although this self-limited adverse event is rare, individuals experiencing this side effect sought another regimen for their second dose, which was AZD1222.

It is possible to mix and match vaccines in specific situations such as a vaccine shortage or adverse reactions following vaccine administration. This study aims to assess the immunogenicity of heterologous inactivated and the adenoviral-vectored COVID-19 vaccine currently available in Thailand to provide preliminary data on their immunogenicity.

2. Methods

2.1. Study design

We performed a cross-sectional study in which leftover sera samples from participants seeking antibody testing following vaccination at the Center of Excellence in Clinical Virology, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University between April and July 2021 were further analyzed. Only samples from participants who received heterologous inactivated and adenoviral-vectored vaccine were used. The inclusion criteria were immunocompetent individuals older than 18 years with no or well-controlled comorbidities and no previous SARS-CoV-2 infection from the medical history. The recommended interval for the heterologous regimen was 3–4 weeks. The study protocol was approved by the Research Ethics Committee of the Faculty of Medicine, Chulalongkorn University (IRB number 491/64). The committee waived the requirement for consent because the samples used were de-identified and anonymous.

We also included two comparison groups vaccinated with the homologous CoronaVac and AZD1222 regimen (IRB no. 192/64, TCTR20210308003) in the analysis. Participants in the comparison groups received CoronaVac or AZD1222 vaccines at the Banphaeo General Hospital, Samutsakorn Province, Thailand, between March and June 2021. Informed consent was obtained during the second-dose vaccination visit. Participants who consented to blood sampling at 21–35 days after full vaccination were scheduled for an extra blood sampling visit at the Banphaeo General Hospital, Samutsakorn Province. The inclusion criteria for the comparison groups were immunocompetent adults above 18 years with no or well-controlled comorbidities and no previous SARS-CoV-2 infection from the medical history. For the homologous AZD1222 group, we excluded 2 individuals who possessed a positive anti-nucleocapsid IgG (>1.4) as the seropositivity could be due to previous infection. For the homologous CoronaVac and heterologous

CoronaVac/AZD1222 groups, participants tested positive for anti-N IgG were not excluded as it was likely to be from a post vaccination with inactivated vaccine.

2.2. Study vaccine

CoronaVac is an inactivated virus vaccine created from African green monkey kidney cells (Vero cells) that have been inoculated with SARS-CoV-2 (CZ02 strain). The virus was inactivated with β -propiolactone and finally absorbed onto aluminum hydroxide. Each vial contains 0.5 mL with 600 SU (equal to 3 μ g) of inactivated SARS-CoV-2 virus as antigen [11]. Chimpanzee adenovirus Oxford 1 (ChAdOx1)-vectored vaccine (AZD1222) from Oxford/AstraZeneca is a non-replicating viral vector vaccine that stimulates an immune response against the coronavirus spike protein. One dose (0.5 mL) contains no less than 2.5×10^8 infectious units of chimpanzee adenovirus encoding the SARS-CoV-2 spike glycoprotein (ChAdOx1-S) [12]. The CoronaVac vaccine was given as a two-dose regimen administered 21 days apart and prioritized for adults aged 18–59 years. AZD1222 was given as a two-dose regimen administered 10 weeks apart for adults ≥ 18 years and prioritized for the elderly above 60 years of age.

2.3. Blood samples

Venous blood samples (5 mL) samples were collected at a variety of timepoints after second dose vaccination in participants who received heterologous inactivated and adenoviral-vectored vaccine. For participants who received homologous CoronaVac and AZD1222 vaccines, venous blood samples (5 mL) were collected at 21–35 days after the second dose vaccination.

2.4. Laboratory testing

All sera samples were tested for SARS-CoV-2 anti-nucleocapsid (anti-N) and anti-RBD IgG by SARS-CoV-2 IgG II Quant assay (Abbott Diagnostics, Sligo, Ireland). This assay quantifies specific IgG against the RBD of the spike protein and nucleocapsid protein by using a chemiluminescent microparticle immunoassay (CMIA). The anti-RBD IgG result was expressed as arbitrary units per milliliter (AU/mL) and the positive cut-off level was ≥ 50 AU/mL. The AU/mL unit was converted to binding antibody units per milliliter (BAU/mL) according to the World Health Organization standard. The anti-N IgG results were used to exclude participants in the homologous AZD1222 group (data not shown).

The neutralizing activity in a subset of serum samples was tested against the original Wuhan strain and variants of concern, B.1.1.7, B.1.617.2 and B.1.351, using an enzyme-linked immunosorbent assay (ELISA)-based surrogate virus neutralization test (sVNT). A subset of samples for sVNT was randomly selected using an online random number generator (<https://stattrek.com/statistics/random-number-generator.aspx>). For the original Wuhan strain, sera samples were diluted and tested following the kit instructions for sVNT (Euroimmun, Lubeck, Germany). The positive cutoff was defined as $\geq 35\%$ inhibition. The sVNT against wild type, B.1.1.7, B.1.351 and B.1.617.2 was tested by cPassTM SARS-CoV-2 neutralizing antibody detection kit (GenScript, Jiangsu, China). The recombinant RBD of the SARS-CoV-2 spike protein contains N501Y for the B.1.1.7 variant, N501Y, E484K, and K417N for the B.1.351 variant, and L452R and T478K for the B.1.617.2 variant. Briefly, the sera samples were diluted 1:10 with sample dilution buffer and then incubated with RBD-horseradish peroxidase (HRP) for 30 min at 37 °C. Then 100 μ L of the sample mixture was subsequently added to a capture plate with pre-coated human

angiotensin-converting enzyme 2 (ACE2) protein and incubated for 15 min at 37 °C. After a washing step, 100 µL of 3,3',5,5'-tetramethylbenzidine (TMB) solution was added and the plate incubated in the dark for 15 min at 20–25 °C. Then, 50 µL of a stop solution was added to quench the reaction and the sample was read immediately at 450 nm. The percent inhibition of a sample was calculated as (1-average optical density (OD) of sample/average OD of negative control) × 100%. Greater than or equal to 30% inhibition was considered indicative of the presence of neutralizing antibodies.

2.5. Statistical analysis

Baseline characteristics were reported in median with interquartile range (IQR). Anti-RBD IgG titers were presented as geometric mean titer (GMT) and 95% confidence interval (CI). The sVNT ACE2-RBD binding percent inhibition was presented as median with interquartile range. Baseline characteristics including potential confounders such as age, sex and presence of comorbidity were adjusted using ANCOVA with Bonferroni correction. Statistical analysis was performed using SPSS v23.0 (IBM Corp, Armonk, NY). Figures were generated using Prism 8.0 (GraphPad, San Diego, CA). A P-value of < 0.05 was considered statistically significant.

3. Results

3.1. Demographic data

All participants were Thai. The median age of participants in the homologous AZD1222 vaccine cohort (median 50 years; IQR 33–64) was significantly higher than that in the homologous CoronaVac (median 43 years; IQR 35–48) and the heterologous CoronaVac/AZD1222 (median 38 years; IQR 31–44) ($P < 0.001$) because of vaccine prioritization during initial implementation (Table 1). Unlike the homologous vaccination cohorts, there were variations in intervals between the first and second dose vaccinations among the heterologous CoronaVac/AZD1222 vaccinees (median: 26 days, IQR: 21–32 days, range 14–72 days). We analyzed the immunogenicity data of the heterologous CoronaVac/AZD1222 vaccinees in two sets. The first set included all available data. The second set included heterologous CoronaVac/AZD1222 vaccinees who received vaccines 14–35 days apart and had their blood collected between 14 and 35 days post-second dose vaccination

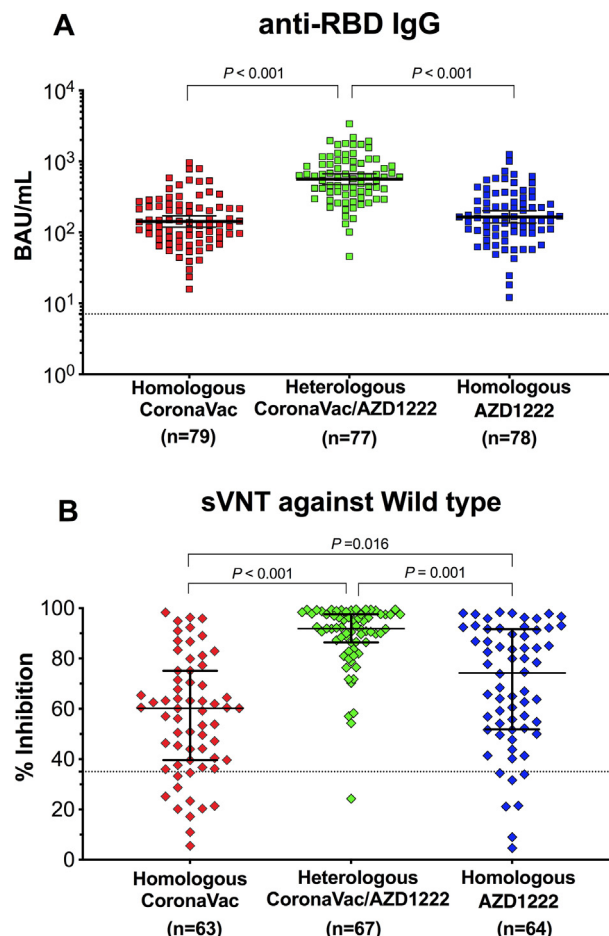


Fig. 1. Anti-RBD IgG and neutralizing capacity measured by a surrogate virus neutralization test (sVNT) against SARS-CoV-2 original Wuhan strain following heterologous CoronaVac/AZD1222 and homologous CoronaVac or AZD1222 vaccination. (A) anti-RBD IgG obtained at 28 days after homologous CoronaVac/CoronaVac (red), 14–75 days after CoronaVac/AZD1222 (green), and 28 days after homologous AZD1222/AZD1222 (blue). (B) Percentage of inhibition against Wuhan strain was tested using a commercially available test kit (Euroimmun, Lubeck, Germany). Lines indicate geometric mean titers and 95% confidence intervals for anti-RBD IgG and median with interquartile range for sVNT. Dotted lines indicate the manufacturer's pre-specified threshold for seropositivity. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Characteristics of participants in the homologous and heterologous schedule of CoronaVac and AZD1222 vaccine.

Characteristics	CoronaVac/CoronaVac (N = 79)	CoronaVac/AZD1222 (N = 77)	AZD1222/AZD1222 (N = 78)	P-value
Sex				0.121 [†]
No. (%)				
Male	32 (40.5%)	20 (26%)	30 (38.5%)	
Female	47 (59.5%)	57 (74%)	48 (61.5%)	
Age (years) median (IQR)	43 (35–48)	38 (31–44)	50 (33–64)	<0.001 [§]
Presence of comorbidities	16 (20.3%)	13 (16.9%)	28 (35.9%)	0.013 [†]
No. (%)				
Interval between 1st and 2nd dose median (IQR)	21 (21–21)	26 (21–32)	70 (70–70)	
[range]	[21–21]	[14–72]	[68–101]	
Interval between 2nd dose and sample collection date median (IQR)	28 (27–28)	31 (29–35)	29 (26–31)	
[range]	[27–32]	[14–75]	[23–35]	

[†] Statistical analyses were performed using Chi-square test.

[§] Statistical analyses were performed using t-test. IQR; interquartile range.

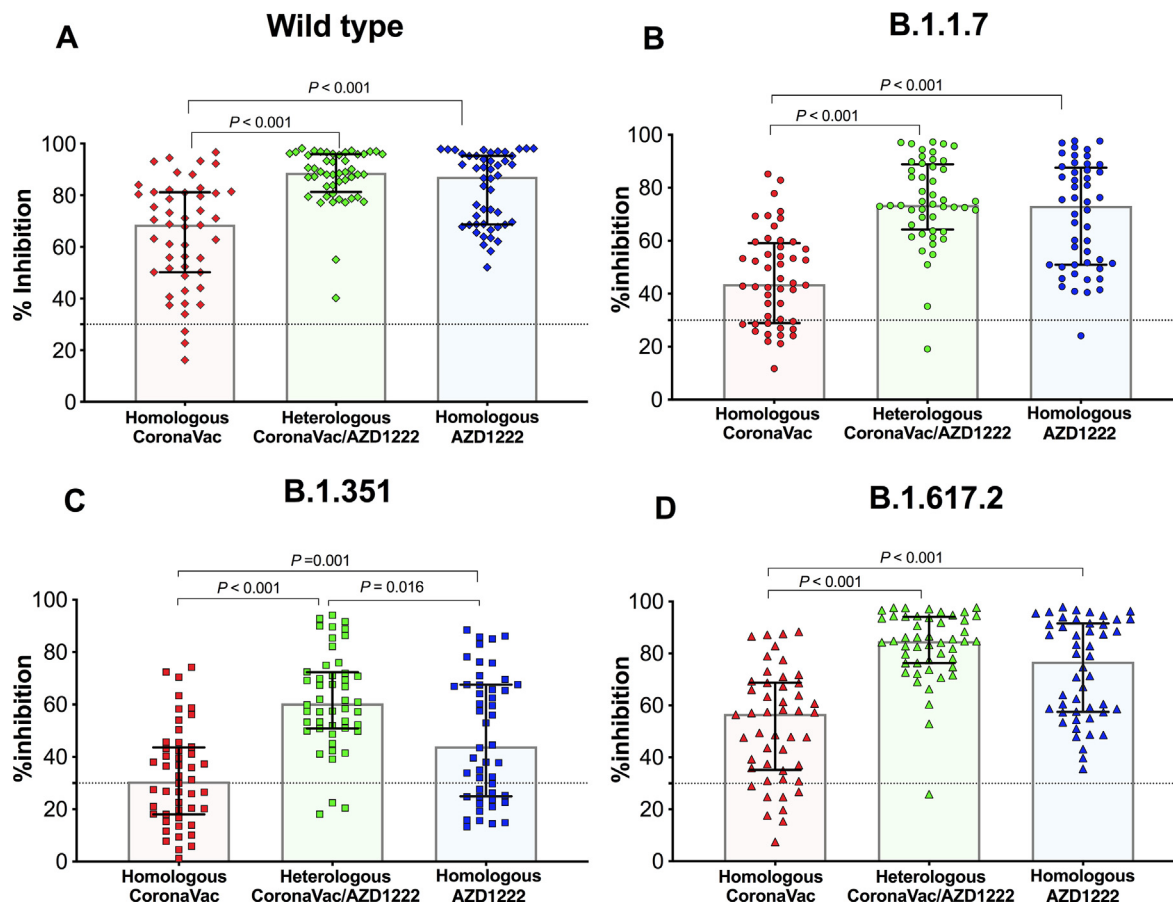


Fig. 2. Neutralizing capacity measured by a surrogate virus neutralization test (sVNT) against SARS-CoV-2 wild-type and variants following heterologous CoronaVac/AZD1222 and homologous CoronaVac or AZD1222 vaccination. Percentage of inhibition against SARS-CoV-2 (A) Wild type (rhombus), (B) B.1.1.7 (circle), (C) B.1.351 (square) and (D) B.1.617.2 (triangle) was measured using cPass™ (GenScript, Jiangsu, China). Bars indicate median. Lines indicate the interquartile range. Dotted lines indicate the manufacturer’s pre-specified threshold for seropositivity.

(Supplementary Fig. 1 and Supplementary Table 1). Differences between all available data and data from participants with similar characteristics among all groups were not significant. Therefore, we presented all available data in the results section.

Apart from age, there was a difference in the presence of comorbidities among the three groups (Table 1). Participants in the homologous AZD1222 group had higher comorbidity rate than the homologous and the heterologous CoronaVac/AZD1222 ($P = 0.013$). Potential confounders including age and presence of comorbidities were taken into account and adjusted in all analyses of this study.

3.2. Anti-RBD RBD IgG and sVNT against wild type

Anti-RBD IgG was detected in 100% of participants in all groups after dose 2, with the GMT (95% CI) of 142.8 (118.8–171.6), 562.6 (472.5–670.0), and 165.6 (135.9–201.8) BAU/mL among the homologous CoronaVac, heterologous CoronaVac/AZD1222 and homologous AZD1222 groups, respectively. CoronaVac followed by AZD1222 vaccine induced higher levels of anti-RBD IgG than two-dose CoronaVac (approximately 3.9-fold) and AZD1222 vaccine (approximately 3.4-fold) ($P < 0.001$) (Fig. 1A).

The sVNT was based on antibody-mediated blockage of ACE2–RBD–protein interaction. The percent inhibition represented the ability of sera from vaccinated individuals to block the interaction between the ACE2 receptor protein and the SARS-CoV-2 RBD,

which reflects the neutralizing capacity. Our results showed that the heterologous CoronaVac/AZD1222 vaccine recipients had higher neutralizing capacity measured by sVNT against the original Wuhan (wild type) (median = 91.9%) than the homologous CoronaVac (median = 60.2%) and AZD1222 vaccine recipients (median = 74.3%) (Fig. 1B).

3.3. Neutralizing capacity against SARS-CoV-2 variants

Our results showed that sera samples of the heterologous CoronaVac/AZD1222 vaccine recipients had higher neutralizing capacity measured by sVNT against the wild-type and all variants of concern than the recipients of the two-dose CoronaVac ($P < 0.001$) (Fig. 2A–D). In addition, the homologous AZD1222 vaccine recipients also had higher neutralizing activities against the wild-type and all variants of concern than in the recipients of the CoronaVac ($P < 0.001$). Comparison of neutralizing activities between the heterologous CoronaVac/AZD1222 and the homologous AZD1222 vaccine recipients showed similar neutralizing capacity against the SARS-CoV-2 wild-type, B.1.1.7 and B.1.617.2 but lower neutralizing capacity against B.1.351 in the homologous AZD1222 group than the heterologous group ($P = 0.016$) after adjustment of age, sex and presence of comorbidities as potential confounders. (Fig. 2C).

Our study showed that there was a reduction in neutralizing capacity of sera against B.1.1.7, B.1.351 and B.1.617.2 compared

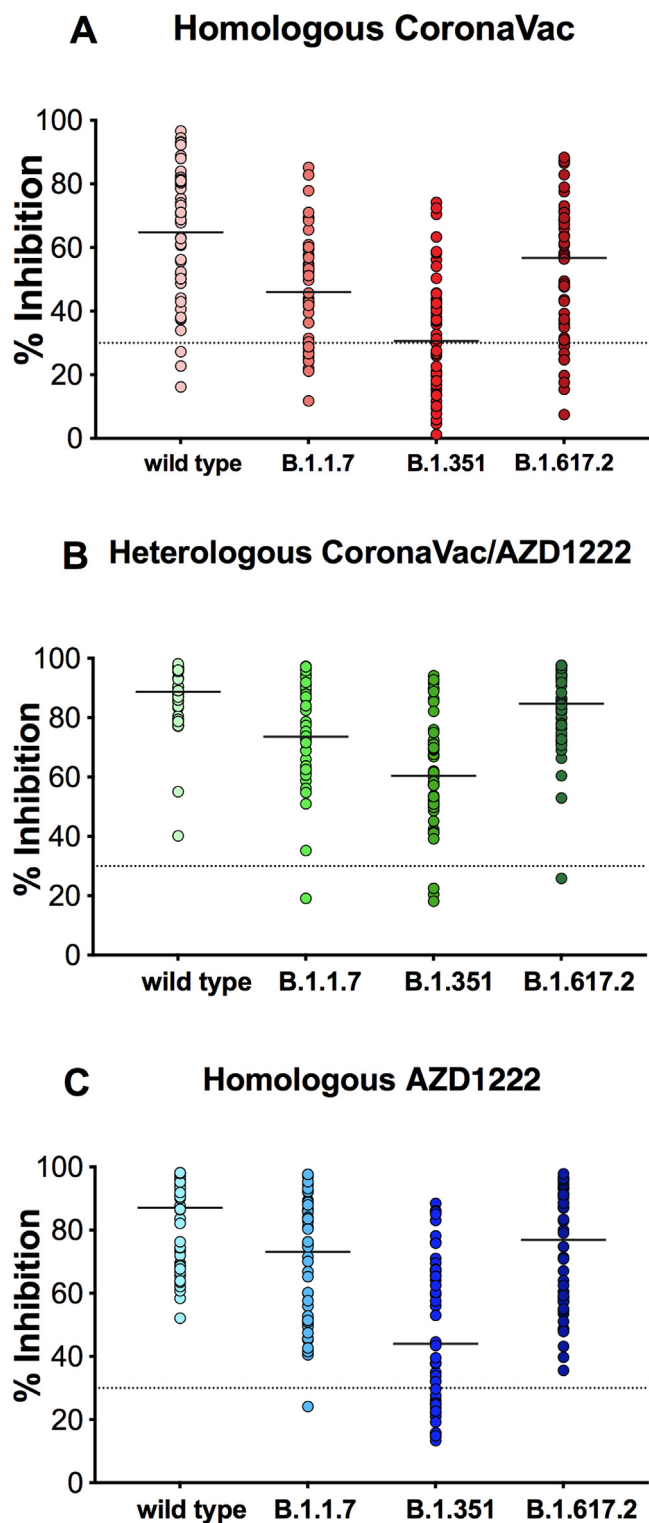


Fig. 3. Comparison between neutralizing capacity measured by a surrogate virus neutralization test (sVNT) against variants relative to the wild type in sera samples obtained from (A) homologous CoronaVac/CoronaVac, (B) heterologous CoronaVac/AZD1222, and (C) homologous AZD1222/AZD1222 vaccinees. Lines indicate the median of percent inhibition. Dotted lines indicate the manufacturer’s pre-specified threshold for seropositivity.

to the wild-type strain in all vaccinated groups (Fig. 3A-C). The variant with markedly reduced capacity was B.1.351.

4. Discussion

As of August 2021, the Department of Disease Control, Ministry of Public Health, Thailand, has implemented policies designating the first vaccine dose to be the CoronaVac vaccine and the second dose to be the AZD1222 vaccine, with more than 300,000 individuals getting vaccinated under this policy. The reasons are the shortage of AZD1222 and the decreased effectiveness of the two-dose CoronaVac vaccine against the SARS-CoV-2 delta variant that are circulating in Thailand.

Our study enrolled recipients who had received heterologous inactivated vaccine (CoronaVac) and adenoviral-vectored COVID-19 vaccine (AZD1222) and sought antibody testing following vaccination, and they were compared with homologous vaccine recipients. Our immunogenicity data has shown that anti-RBD IgG was detected in 100% of heterologous CoronaVac/AZD1222 recipients after the second dose, with a higher GMT than those elicited by the two-dose CoronaVac and AZD1222 vaccines after adjusting for age, sex and presence of comorbidities. In addition, the sera samples of the CoronaVac/AZD1222 vaccine recipients had higher neutralizing capacity against the original Wuhan, B.1.1.7, B.1.617.2 and the B.1.351 strain than did the recipients of two-dose CoronaVac. Although the extent of the efficacy of the heterologous regimen has not been studied, the comparatively high level of immunogenicity compared to the homologous regimen supports its use as an alternative schedule.

Researchers are investigating to determine the immune correlates of protection to use as surrogate endpoints for vaccine efficacy. In a recent study, binding and neutralizing antibodies were correlated with a reduced risk of symptomatic SARS-CoV-2 infection, for example, anti-RBD IgG level of 506 BAU/mL correlated to the vaccine efficacy of 80% against symptomatic infection with Alpha (B.1.1.7) variant [13]. Because of its ability to elicit a high anti-RBD IgG and neutralizing capacity following two-dose vaccination, a heterologous regimen with CoronaVac/AZD1222 may provide better protection than the homologous CoronaVac regimen. Regarding the reactogenicity of the heterologous regimen, we did not record the adverse events following homologous and heterologous vaccination in this cohort. Nevertheless, our previous prospective cohort study showed that the heterologous CoronaVac/AZD1222 regimen resulted in a higher percentage of local and systemic adverse events compared to the homologous group after the second dose vaccination which is in agreement with the initial reactogenicity data in the Com-COV trial [3,8].

Several SARS-CoV-2 variants such as B.1.351 and B.1.671.2 have demonstrated their ability to evade vaccine-induced immunity in the context of reducing the vaccine-induced neutralizing capacity and decreased vaccine effectiveness against symptomatic infection [14–15]. A recent study has shown that two doses of AZD1222 had an efficacy of 10.4% against the B.1.351 [16]. Our study also showed a reduction in neutralizing capacity of sera against B.1.1.7, B.1.167.2 and B.1.351 compared to the original wild-type strain. Nevertheless, the neutralizing capacity against the Delta variant B.1.617.2 among the heterologous vaccine recipients was higher than the homologous CoronaVac group. This result implied an additional benefit against variants of concern in the heterologous CoronaVac/AZD1222 regimen compared to the two-dose CoronaVac regimen.

The high immunogenicity of the heterologous CoronaVac/AZD1222 regimen in this study is congruent with conclusions of another heterologous CoronaVac/ adenovirus type-5-vectored Convidecia study [17]. Heterologous administration with adenovirus type-5-vectored vaccine in CoronaVac-primed individuals resulted in improved immunogenicity in both humoral (neutraliz-

ing antibody and anti-RBD IgG) and cellular responses (T helper-1-biased response) with an increased in the occurrence of solicited injection site reactions than those receiving the homologous CoronaVac. Heterologous vaccination regimens have been previously examined with experimental vaccines for HIV [18], malaria [19], and Ebola [20], a precedent for this regimen; however, the mechanism for increased immunogenicity from mixing CoronaVac/AZD1222 has yet to be elucidated.

There are a few noteworthy limitations to the current study. Because our study participants were recruited in a real-world setting before the Ministry of Public Health launched the vaccine recommendation, the schedule of heterologous vaccination did not follow the recently released guideline stating that two doses should be given 28 days apart. In this study, approximately 80.5% of individuals received the first and second dose of the heterologous regimen at an interval between 14 and 35 days. Secondly, the timing of blood collection after the second dose in the heterologous group was not the same as in the homologous group. There might be residual confounding due to differences in dosing and blood collection intervals. Third, age and presence of comorbidities demographic disparity was present between different vaccination regimen groups. The inherent nature of Thailand's vaccination policy, which prioritizes vaccination with the AZD1222 vaccine in elderly people, consequently led to a higher average age for the homologous AZD1222 regimen cohort. A previous AZD1222 randomized trial showed that anti-spike IgG responses decreased with increasing age after the first dose but not the second dose vaccination [21]. The increased average age in the homologous AZD1222 regimen cohort may lead to a lower immune response as also demonstrated in a study of immunogenicity of an mRNA vaccine [22]. The age-related lower immune response is likely due to the "immunosenescence" phenomenon as a result of increases in terminally differentiated memory cell populations, lymph node fibrosis, and altered cytokine production among the elderly [23]. Nevertheless, all of the potential confounders including age, sex and presence of comorbidities were adjusted. This study had a small sample size. Additional studies on a larger group of individuals are required to determine the immunogenicity of the heterologous vaccine in different settings. A surrogate viral neutralization test used in this study is a robust serological test to detect neutralizing capacity to SARS-CoV-2 with 99.93% specificity and 95–100% sensitivity [24] but it could not be directly compared with the live virus neutralization test in other studies. Lastly, cell-mediated immunity was not explored.

In conclusion, a heterologous CoronaVac/AZD1222 regimen demonstrated a robust anti-RBD IgG and neutralizing antibody response. The heterologous regimen with CoronaVac followed by AZD1222 would provide greater flexibility for countries experiencing supply difficulties and individuals with adverse events following the first dose CoronaVac vaccination.

Data availability

All data generated during this study are contained within this manuscript and its [Supplementary Information](#) files.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.vaccine.2022.04.043>.

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