Immunogenicity of heterologous prime/booster-inactivated and adenoviral-vectored COVID-19 vaccine: real-world data

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Article

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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 prime/booster-inactivated COVID-19 vaccine and adenoviral-vectored vaccine were enrolled in our study. There were two comparison groups vaccinated with the homologous CoronaVac (N = 79) and AZD1222 (N = 80) regimen. All sera samples were tested for SARS-CoV-2 spike receptor-binding-domain (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 and B.1.351, using an enzyme-linked immunosorbent assay (ELISA)-based surrogate virus neutralization test (sVNT). The CoronaVac followed by the AZD1222 vaccine induced higher levels of spike RBD-specific IgG than that of two-dose 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 the B.1.351 strain than in the recipients of the two-dose CoronaVac or AZD1222. Following the inactivated CoronaVac/adenoviral-vectored (AZD1222) vaccination administered 14–72 days apart, participants receiving the heterologous vaccine regimen had higher spike RBD-specific IgG and neutralizing activities than the homologous CoronaVac vaccine recipients.

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 mRNA vaccines induced higher serum anti-spike receptor-binding domain (RBD) and neutralizing antibody titers than the homologous efficacy-proven adenoviral-vectored two-dose regimens[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].

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)[3]. The preliminary results showed that the heterologous vaccine regimen (AZD1222/BNT162b2) induced higher humoral and cellular responses to SARS-CoV-2 compared to the homologous vaccine regimen (AZD1222/AZD1222)[4]. 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 symptomatic COVID-19 following two-dose CoronaVac vaccination[5-7]. Health professionals were the first prioritized group receiving two-dose CoronaVac in Thailand. 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. Preliminary studies have found the two-dose CoronaVac regimen induced a lower, but acceptable, immune response compared to the two-dose AZD1222 regimen; however, there was a significantly shorter waiting period between CoronaVac doses. Thailand started vaccinations with the inactivated CoronaVac on 28 February, 2021, and AZD1222 vaccine on 16 March, 2021, with a 3- to 4-week and 10-week waiting period between doses, respectively. A 10-week waiting period 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[8]. The CoronaVac vaccine was associated with a rare focal neurological syndrome characterized by numbness, or sometimes weakness, in the limbs[9]. Although this self-limited adverse event is rare, individuals experiencing this side effect sought another regimen for their second shot, which was AZD1222.

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

Results

3.1 Demographic data

All participants were Thai; however, the mean age of participants in the homologous AZD1222 vaccine cohort was higher than that in the homologous CoronaVac and heterologous CoronaVac/AZD1222 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). 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 (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.

Table 1. Characteristics of the participants in the homologous and heterologous prime/booster of CoronaVac and AZD1222 vaccine.

Characteristics	CoronaVac/CoronaVac (N = 79)	CoronaVac/AZD1222 (N = 77)	AZD1222/AZD1222 (N = 80)
Sex No. (%)			
Male	32 (40.5%)	20 (26%)	31 (38.8%)
Female	47 (59.5%)	57 (74%)	49 (61.2%)
Age yrs, mean (SD)	42 (9.7)	38.66 (9.6)	48.1 (17.8)
Underlying disease No. (%)	19 (24.1%)	13 (16.9%)	48 (60%)
Diabetes mellitus	4	1	15
Hypertension	8	8	20
Heart disease	1	2	3
Other health conditions	6	2	10
Interval between 1st and 2nd dose median (IQR)	21	26 (21-32)	70
Interval between 2nd dose and collection date median (IQR)	28 (27-28)	31 (29-35)	29 (26-31)

3.2 Receptor-binding domain (RBD)-specific IgG and sVNT

RBD-specific IgG was detected in 100% of participants in all groups after dose 2, with the GMT (95% CI) of 1006 (836.8-1209), 1207 (989.4-1472), and 3962 (3327-4718) AU/ml among the homologous CoronaVac, homologous AZD1222, and heterologous CoronaVac/AZD1222 groups, respectively. CoronaVac followed by AZD1222 vaccine induced higher levels of spike receptor-binding domain-specific IgG than two-dose CoronaVac and AZD1222 vaccines (P<0.001) (Fig 1A).

The sVNT was based on antibody-mediated blockage of ACE2–spike protein–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 activity. Our results showed that CoronaVac/AZD1222 vaccine recipients had higher neutralizing activities against the original Wuhan and the B.1.351 strain than the recipients of two-dose CoronaVac and AZD1222 (P<0.01) (Fig. 1B and D, respectively). In addition, the CoronaVac/AZD1222 vaccine recipients had higher

neutralizing activities against the B.1.1.7 strain than the recipients of two-dose CoronaVac but were comparable to those in the recipients of two-dose AZD1222 (Fig. 2C) (P<0.01).

3.3 Lower neutralizing activities against SARS-CoV-2 variants

As compared to the percentage of neutralizing activity against the original Wuhan strain, we found that neutralizing activities of sera against B.1.1.7 and B.1.351 were approximately 1.25-1.5 times and 1.5-1.97 times lower than that against the original strain (Fig. 2). The decline of neutralizing activity against the two variants (1.5 x for B.1.1.7 and 1.97 x for B.1.351) was highest among those who received the homologous CoronaVac/CoronaVac vaccine (P<0.01) (Fig 2A). Although there was a reduction in neutralizing activities of sera against B.1.1.7 (1.25x) and B.1.351 (1.5x) in heterologous CoronaVac/AZD1222 vaccinees (Fig. 2B), a higher level than was observed compared to the homologous CoronaVac/CoronaVac counterparts. A similar trend was found between the individuals who received the homologous AZD1222/AZD1222 and heterologous CoronaVac/AZD1222 vaccine (Fig. 2C).

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 200,000 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 variants of concern that are circulating in Thailand.

Our study enrolled recipients who had received heterologous prime/booster inactivated COVID-19 vaccine and adenoviral-vectored vaccine and sought antibody testing following vaccination, and they were compared with homologous vaccine recipients. Our immunogenicity data has shown that RBD-specific 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. In addition, the CoronaVac/AZD1222 vaccine recipients had higher amounts of neutralizing activities against the original Wuhan and the B.1.351 strain than did the recipients of two-dose CoronaVac and AZD1222. Although the extent of the efficacy of the heterologous regimen has not been studied, the comparatively high level of immunogenicity compared to the homologous AZD1222 regimen supports its use as an alternative schedule, with the added benefit of a shorter waiting period between doses.

Researchers are investigating to determine the immune correlates of protection to use as surrogate endpoints for vaccine efficacy. In a recent preprint, protection against SARS-CoV-2 challenge in vaccinated non-human primates strongly correlated with levels of anti-S antibody binding and neutralizing activity[10]. Because of its ability to elicit a high RBD-specific IgG and neutralizing activity following two-dose vaccination, a heterologous prime/booster regimen with CoronaVac/AZD1222 may provide more protection than the homologous CoronaVac regimen. Regarding the reactogenicity of the heterologous regimen, 1,100 individuals received the heterologous CoronaVac/AZD1222 as reported by

Ministry of Public Health, Thailand, between March and July, 2021. However, this retrospective study has not noted any subsequent serious adverse events reported in the National Vaccine Adverse Event Reporting System[11].

Several SARS-CoV-2 variants such as B.1.351 and B.1.671.2 have demonstrated their ability to evade vaccine-induced immunity[12]. A recent study has shown that two doses of AZD1222 had an efficacy of 10.4% against the B.1.351[13]. Our study showed that although there was a reduction in neutralizing activities of sera against B.1.1.7 (1.25 times) and B.1.351 (1.5 times) from the original Wuhan strain in heterologous CoronaVac/AZD1222 vaccinees, neutralizing activities were higher than in the homologous CoronaVac/CoronaVac counterparts. This result implied an additional benefit against variants of concern in the heterologous CoronaVac/AZD1222 regimen.

The high immunogenicity profile of the heterologous prime/booster regimen in this study is congruent with conclusions of the Com-COV study, which also found an increase in anti-spike RBD-specific IgG and neutralizing titers compared to the heterologous regimen[1]. In the Com-COV study, the researchers investigated combinations of the Pfizer mRNA vaccine (BNT162b2) and the AZD1222 vaccines available in the United Kingdom. Two-dose AZD1222 administered 6 weeks apart elicited a lower immune response than when given 10–12 weeks apart. The underlying mechanism is likely due to the host anti-adenoviral antibodies elicited by the first vaccination preventing the virus in the second vaccine dose to enter the cells when the second dose is given sooner than 10–12 weeks. Nevertheless, the Pfizer mRNA vaccine (BNT162b2) given as a second dose in AZD1222-primed individuals has been shown to induce a higher response than that of the AZD1222 given as a second dose. Heterologous vaccination regimens have been previously examined with experimental vaccines for HIV[14], malaria[15], and Ebola[16], a precedent for this regimen's efficacy; 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 collecting blood samples after the second dose in the heterologous vaccine group was not the same as for the homologous vaccine group. This caveat has not statistically affected the conclusion but nonetheless should be considered. Third, the age 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. The increased average age in the homologous AZD1222 regimen cohort can lead to a lower immune response as also demonstrated in a study of immunogenicity of an RNA vaccine[17]. 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[18]. Lastly, cell-mediated immunity was not explored in this study.

In conclusion, a heterologous prime/booster regimen demonstrated a strong immune response. Further studies are underway to determine the reactogenicity and immunogenicity of a heterologous prime/booster regimen in clinical trials against other SARS-CoV-2 variants. With an acceptable immunogenicity profile, here is the first report to show that a heterologous prime/booster regimen with CoronaVac/AZD1222 would provide greater flexibility for countries experiencing supply difficulties and individuals with adverse events following the first dose CoronaVac vaccination.

Materials And 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 prime/booster inactivated COVID-19 vaccine and adenoviral-vectored vaccine were used. The study protocol was approved by the Research Ethics Committee of the Faculty of Medicine, Chulalongkorn University (IRB number 491/64) and this trial is registered with Thai Clinical Trials Registry (TCTR20210628005). 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, TCTR20210319003) 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.

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 μg) of inactivated SARS-CoV-2 virus as antigen[19]. 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)[20]. 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 various time points after second dose vaccination in participants who received heterologous prime/booster inactivated COVID-19 vaccine 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 spike 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 by using a chemiluminescent microparticle immunoassay (CMIA). The result was expressed as arbitrary units per milliliter (AU/mL) and the positive cut-off level was ≥ 50 AU/mL (upper limit: 40,000 AU/mL). Results of this assay had a good correlation with neutralizing antibodies[21].

The neutralizing activity in a subset of serum samples was tested against the original Wuhan strain and variants of concern, B.1.1.7 and B.1.351, using an enzyme-linked immunosorbent assay (ELISA)-based surrogate virus neutralization test (sVNT). For the original Wuhan strain SARS-CoV-2, sera samples were diluted and tested following the kit instructions for sVNT (Euroimmun, Lubeck, Germany). The positive cutoff was defined as ≥ 35% inhibition. The sVNT against B.1.1.7 and B.1.351 used cPass™ SARS-CoV-2 neutralizing antibody detection kit (GenScript, Jiangsu, China). The recombinant RBD of the SARS-CoV-2 spike protein contains N501Y, del (69–70), D614G, P681H, N439K, del Y144, A222V, and A570D for the B.1.1.7 variant and N501Y, E484K, and K417N for the B.1.351 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[22]. Then 100 μL of the sample mixture was subsequently added to a capture plate with pre-coated h-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 20% inhibition was considered indicative of the presence of neutralizing antibodies.

2.5 Statistical analysis

Baseline characteristics are reported as means and standard deviations (SD). IgG S1/RBD antibodies titers are presented as geometric mean titer (GMT) and 95% confidence interval (CI). The sVNT antibodies titers are presented as median with interquartile range. The difference of spike RBD-specific IgG and percentage of inhibition between groups was calculated using the Mann-Whitney U test. The Wilcoxon site rank test was used to compare the different percentages of inhibition between wild type and variants.

Statistical analysis was done using Prism 8.0 (GraphPad, San Diego, CA). A *P*-value < 0.05 was considered statistically significant.

Data Availability

All data generated during this study are contained within this manuscript and its Supplementary Information files.

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Figures

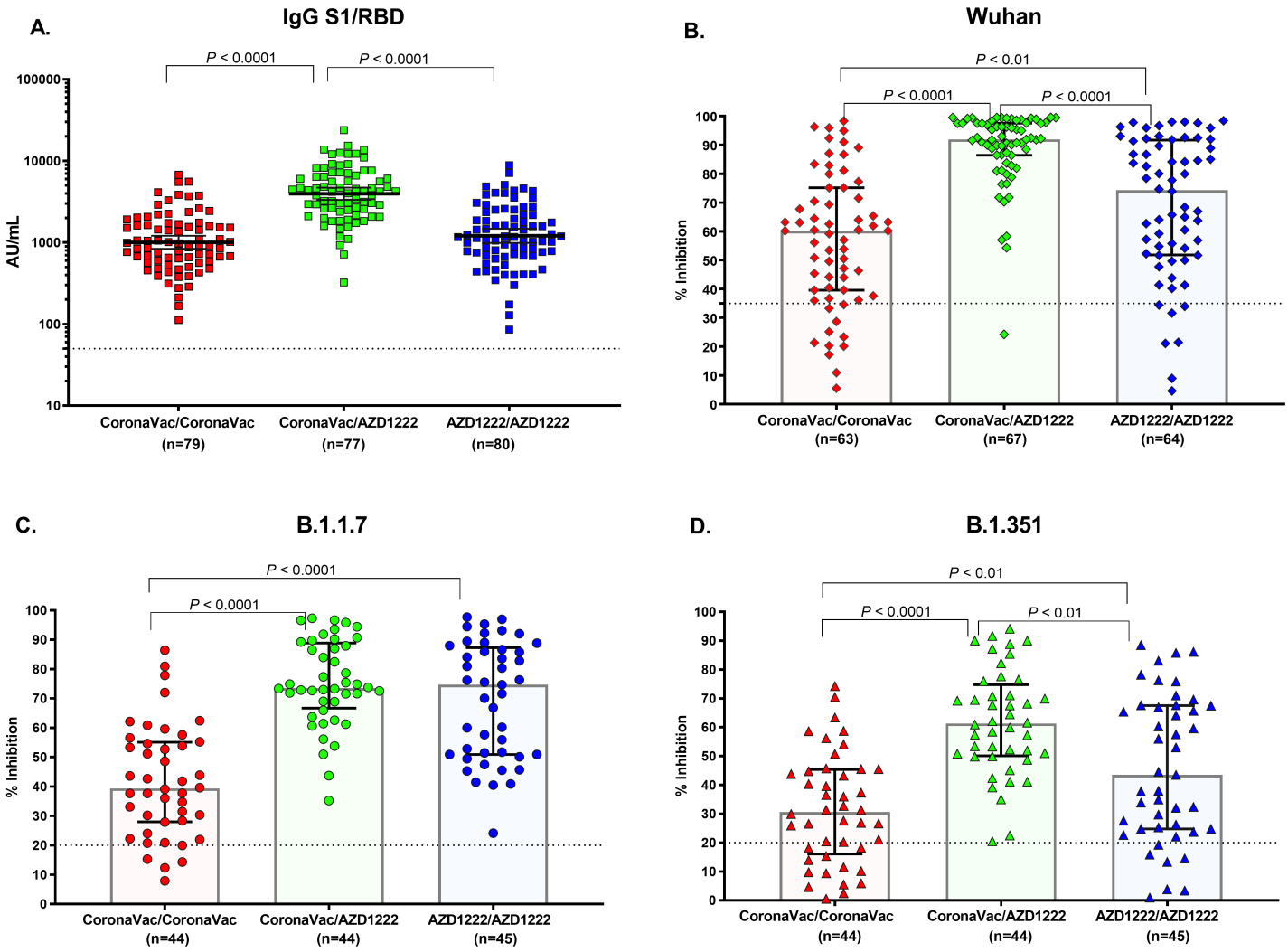

Figure 1

RBD-specific IgG antibodies and neutralizing activities against SARS-CoV-2 original Wuhan strain and variants following heterologous CoronaVac/AZD1222 and homologous CoronaVac or AZD1222 vaccination. (A) RBD-specific IgG titers obtained at 28 days after homologous CoronaVac/CoronaVac (red), 14-72 days after CoronaVac/AZD1222 (green), and 28 days after homologous AZD1222/AZD1222 (blue). Percentage of inhibition against (B) Wuhan (rhombus), (C) B.1.1.7 (circle), and (D) B.1.351 (triangle) SARS-CoV-2 measured using a surrogate virus neutralization test (sVNT). Bars represent GMT (95% CI) for RBD-specific IgG and median (IQR) for sVNT.

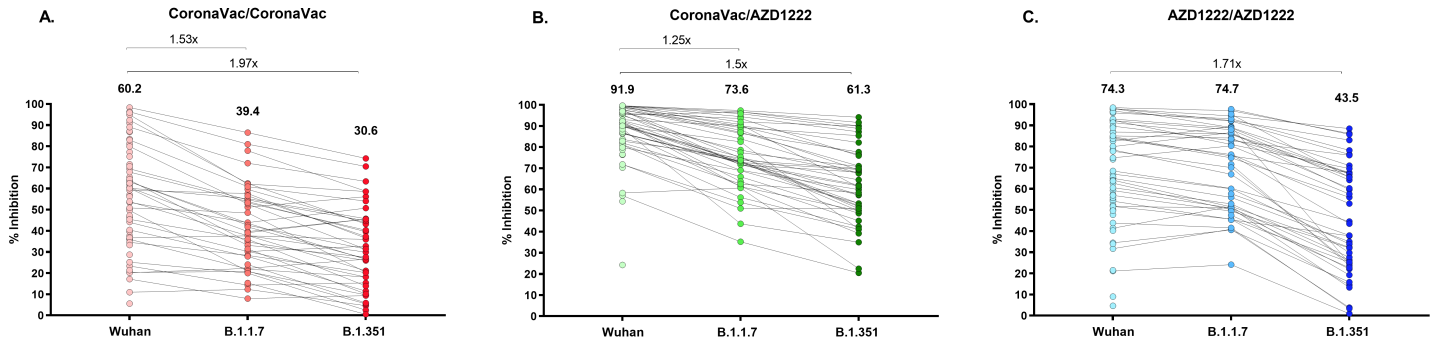

Figure 2

The difference in neutralizing activities against variants relative to the original Wuhan strain. The results are shown as the percent inhibition against the original Wuhan strain and variants of matched serum samples obtained from (A) homologous CoronaVac/CoronaVac, (B) heterologous CoronaVac/AZD1222, and (C) homologous AZD1222/AZD1222 vaccinees. The ratio of percent inhibition against the variants as compared with the original Wuhan strain is labeled above each pair. The number over the aligned plot indicates the median of % inhibition.

Supplementary Files

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- S1Fig.tif
- SupplementaryTable1.docx