IMMUNOGENICITY OF A HETEROLOGOUS INACTIVATED AND mRNA COVID-19 COMBINATION VACCINE REGIMEN

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Abstract. Vaccine shortages and side effects have caused many people to have received a mixed anti-corona virus vaccine regimen using two different types of vaccine (heterologous regimen). In this cross-sectional study, we aimed to evaluate the immunogenicity among healthcare professionals of the following vaccine regimens: a combination of the CoronaVac vaccine (CV) followed by the BNT162b2 vaccine (BNT) (CV/BNT) (n = 76) compared to the two-dose CV regimen (CV/CV) (n = 170) or a two-dose BNT vaccine regimen (BNT/BNT) (n = 19) in order to determine if the CV/BNT regimen may be considered as an alternative to the CV/CV or BNT/BNT regimens. Subjects received the CV/BNT regimen, at short-intervals (21-28 days apart) or long-intervals (≥7 weeks apart). We obtained the following from each subject: serum total immunoglobulin (Ig), immunoglobulin G (IgG) and A (IgA) levels against the receptor-binding domain (RBD) of the SARS-CoV-2 spike (S) protein (anti-RBD Ig, anti-RBD IgG and anti-S IgA (reported as a sample-to-calibrator (S/C) relative light unit ratio), respectively) 21-35 days after the second dose of their vaccine and calculated the geometric mean titer (GMT) (95%CI) for these levels. We evaluated the neutralization activity (NA) of each sample using an ELISA-based surrogate virus neutralization test (sVNT) against wild-type severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and the following SARS-CoV-2 variants: B.1.1.7 (Alpha), B.1.351 (Beta), B.1.617.2 (Delta) and BA.4/5 (Omicron). A total of 265 subjects were included in the study, 65% female. The mean (±standard deviation) age of study subjects was 37 (±12) (range: 14-59) years. The GMT (95%CI) of the long and short interval total anti-RBD Ig levels elicited by the CV/BNT regimen against the wild-type

SARS-CoV-2 variant were 1,042 (828-1,311) and 10,485 (7,228-15,209) U/ml, the anti-RBD IgG were 1,475 (1,267-1,716) and 2,683 (2,075-3,469) BAU/ml and the median (IQR) anti-S IgA S/C ratios were 6.6 (4.9-9.0) and 9 (7.8-9.0, respectively. The GMT (95%CI) of total anti-RBD Ig level elicited by the CV/CV regimen against the wild-type SARS-CoV-2 variant was 98 (83-116) U/ml, the anti-RBD IgG was 128 (114-144) BAU/ml and the median (IQR) anti-S IgA S/C ratio was 0.9 (0.6-1.8). The GMT (95%CI) of the total anti-RBD Ig level elicited by the BNT/BNT regimen against the wild-type SARS-CoV-2 variant was 1,963 (1,378-2,798) U/ml, the anti-RBD IgG was 1,651 (1,182-2,306) BAU/ml, and the median (IQR) anti-S IgA S/C ratio was 5.6 (4.5-8.4). The GMT of the total anti-RBD Ig levels elicited by the short- and long-interval CV/BNT regimens were significantly higher (<0.001, <0.001) than the CV/CV regimen, and significantly higher (0.019, <0.001) than the BNT/BNT regimen, respectively. The GMT of the anti-RBD IgG levels elicited by the short- and long-interval CV/BNT regimen were significantly higher (<0.001, <0.001) than the CV/CV regimen but not significantly different (0.814 and 0.774) than the BNT/BNT regimen, respectively. The median (IQR) NA elicited by the short-interval CV/BNT regimen against the wild-type, Alpha, Beta, Delta and Omicron variants were 96 (93-97), 85 (79-89), 77 (69-83), 93 (89-95), and 27 (22-35), respectively, the long-interval CV/BNT regimen were 97 (97-98), 98 (97-98), 95 (94-96), 97 (97-98), and 54 (38-84), respectively, the CV/CV regimen were 67 (49-79), 42 (29-58), 35(21-47), 49 (36-63), and 11 (9-15) and the BNT/BNT regimen were 97 (96-98), 94 (88-95), 89 (80-91), 97 (95-98) and 33 (27-42), respectively. The median NA levels against the Omicron variant elicited by the short- and long-interval CV/BNT regimen were significantly higher (<0.001, <0.001) than the CV/CV regimen but not significantly different (0.416, 0.492) than the BNT/BNT regimen. In summary, the CV/BNT regimen provided significantly higher anti-RBD IgG levels than the CV/CV regimen and similar levels than the BNT/BNT regimen. We conclude the CV/BNT regimen may be a reasonable alternative for initial vaccination. Further studies are needed to determine the long-term effects of these regimens.

Keywords: heterologous, inactivated, mRNA, COVID-19, vaccine, SARS-CoV-2

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INTRODUCTION

Vaccine shortages and side effects have caused many people to have received a mixed anti-corona virus vaccine regimen using two different types of vaccine (heterologous regimen). A clinical trial of a heterologous chimpanzee adenovirus-vectored vaccine (ChAdOx-1) followed by a messenger RNA (mRNA) BNT162b2 vaccine combination (CombiVacS) was conducted in Europe in April 2021 due to concerns about thrombotic events among two-dose ChAdOx-1 vaccine recipients (Borobia *et al*, 2021). The results showed the heterologous ChAdOx-1/BNT162b2 vaccine regimen was significantly more immunogenic than the homologous ChAdOx-1/ChAdOx-1 vaccine regimen.

During March-July 2021, Thailand only had 2 coronavirus disease 2019 (COVID-19) vaccines available: the inactivated CoronaVac (CV) and the ChAdOx-1 vaccine. In August 2021, the BNT162b2 mRNA COVID-19 vaccine (BNT) became available in Thailand. Due to a limited supply of the BNT vaccine, it was first given to healthcare workers (HCW). The BNT vaccine regimen for HCWs consisted of two doses given three weeks apart (BNT/BNT). For HCWs who had received the two-dose inactivated CV vaccine (CV/CV), a third dose of BNT was given as a booster. HCWs who had initially received the CV or adenoviral-vectored (ChAdOx1-S) vaccine for their first dose, received the BNT vaccine as a second dose, irrespective of the time interval between the first and second dose.

In this study we aimed to compare the immunogenicity of a CV/BNT regimen with CV/CV and BNT/BNT regimens in order to determine if CV/BNT might be considered as an alternative regimen to the CV/CV and BNT/BNT regimens.

MATERIALS AND METHODS

Study cohort

Study subjects were HCWs who received a CV/BNT, CV/CV or BNT/BNT vaccine regimen and had requested antibody testing at the research unit of the Center of Excellence in Clinical Virology, Faculty of Medicine, Chulalongkorn University 21-35 days after the second dose of their vaccine regimen.

Vaccination and blood collection

The CV vaccine (Sinovac Biotech Ltd, Beijing, China) was created from African green monkey kidney

cells (Vero cells) inoculated with the CZ02 strain of the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) (Benjamanukul *et al*, 2022). Each 0.5 ml dose contained 3 μg of inactivated SARS-CoV-2 whole virus as the antigen (Benjamanukul *et al*, 2022). The CV/CV regimen dosages were administered 2-4 weeks apart as recommended by the World Health Organization (Jin *et al*, 2022). The BNT162b2 Pfizer BioNTech vaccine (Pfizer Inc, Philadelphia, PA) is a messenger (mRNA) vaccine and each dose contained 30 μg of tozinameran, a lipid nanoparticle encapsulating the modified (Polack *et al*, 2020). The BNT/BNT dosages were administered 3 weeks apart as recommended by the Advisory Committee on Immunization Practices (Dooling *et al*, 2021). The dosages of the CV/BNT regimen were administered at varying intervals divided into 2 interval groups: 21-28 days apart and ≥7 weeks apart.

Antibody assays

Serum was obtained from each study subject and examined for: 1) total immunoglobulin (Ig) level against the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein (anti-RBD Ig) using the Elecsys SARS-CoV-2 S (Roche Diagnostics, Basel, Switzerland); 2) anti-RBD IgG levels using the SARS-CoV-2 IgG II Quant assay (Abbott Diagnostics, Abbott Park, IL); and 3). anti-spike protein 1 (S1) IgA using an enzyme-linked immunosorbent assay (ELISA) (Euroimmun, Lübeck, Germany). The results of the anti-S1 IgA tests were reported as a sample-to-calibrator (S/C) relative light unit ratio; a S/C ratio ≥1.1 was considered to be reactive. An anti-S1 IgA S/C ratio >9 was recorded as a ratio of 9.

Neutralizing activity was determined against the following severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) serotypes: Wuhan (wild-type), B.1.1.7 (alpha), B.1.351 (beta), B.1.617.2 (delta) and BA.4/5 (omicron) with an ELISA-based surrogate virus neutralization test (sVNT) (cPass™, GenScript Biotech, Piscataway, NJ) as described previously (Wanlapakorn *et al*, 2022b). The ability of a serum sample to inhibit binding between RBD and angiotensin-converting enzyme 2 (ACE2) was calculated as percentage using the following equation: 1- (the average optical density of the studied sample/ the average optical density of the negative control) × 100.

Statistical analysis

Baseline characteristics were reported as means (± standard deviations (SD)) or medians (interquartile ranges (IQR)). Total anti-RBD Ig and anti-RBD IgG levels are presented as a geometric mean titer (GMT) with a 95% confidence interval (CI). Other antibody levels and percent of inhibition are presented as medians (IQR). The total anti-RBD Ig and IgG levels were log10 transformed. The differences in log-transformed total anti-RBD Ig and IgG levels by group were analyzed using the analysis of covariance (ANCOVA) with a Bonferroni adjustment and the potential confounders of age and sex were adjusted for. Comparisons of anti-S1 IgA S/C ratios and neutralizing activities among the SARS-CoV-2 variants were made using the Kruskal-Wallis method with Dunn's multiple comparisons. Statistical analysis was performed using the Statistical Package of Social Sciences (SPSS), version 21.0 (IBM Corp, Armonk, NY). Figures were generated using GraphPad Prism, version 9.0 (GraphPad, San Diego, CA). A p-value of <0.05 was considered statistically significant.

Ethical considerations

This study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice principles and was approved by the Research Ethics Committee of the Faculty of Medicine, Chulalongkorn University (IRB numbers 870/64 and 192/64). All subjects gave written informed consent prior to inclusion in the study.

RESULTS

Demographic data

A total of 265 subjects were included in the study: 63 (96% female) who received the CV/BNT regimen, 8 participants (80% female) in the CV/BNT long-interval group, 89 (52% female) who received the CV/CV regimen and 12 (63% female) who received the BNT/BNT regimen (Table 1). The mean (±SD) (range) ages of study subjects who received the short-interval CV/BNT, long-interval CV/BNT, CV/CV and BNT/BNT regimens were: 37 (±12) (range: 14-59), 24 (±6) (range: 20-49), 31 (±11) (range: 20-57), 42 (±6) (range: 18-59), and 34 (±12) (range: 14-54) years, respectively.

Total anti-RBD Ig and IgG levels

The GMT (95%CI) total anti-RBD Ig levels among subjects who received the short- and long-interval CV/BNT

Table 1

Characteristics of study subjects

Characteristics	Vaccine received and number of participants who received such vaccine				
	Total (n = 265)	CV/CV (n = 170)	Short-interval CV/BNT (n = 66)	Long-interval CV/BNT (n = 10)	BNT/BNT (n = 19)
Gender: Female, n (%)	172 (65)	89 (53)	63 (96)	8 (80)	12 (63)
Age in years, mean ± SD (range)	37 ±12 (14-59)	42 ±10 (18-59)	24 ±6 (20-49)	31 ±12 (20-57)	34 ±12 (14-54)
Median [IQR] (range) interval in days between the 1st and 2nd doses	21 [21, 26] (21-156)	23 [21, 26] (21-28)	21 [21, 21] (21-26)	108 [78,138] (50-156)	21 [21, 24] (21-31)
Median [IQR] (range) interval in days between the 2nd dose and blood collection	30 [27, 31] (21-49)	29 [27, 31] (27-49)	29 [29-35] (26-35)	31[29, 35] (29-35)	34 [31, 35] (22-35)

BNT: mRNA BNT162b2 COVID-19 vaccine (Pfizer-BioNTech Inc, New York City, NY); BNT/BNT: 2 doses of the BNT162b2; CV/CV: 2 doses of the CoronaVac vaccine (Sinovac Life Sciences Co Ltd, Beijing, China); CV/BNT: CoronaVac (1st dose) then BNT162b2 (2nd dose); IQR: interquartile range; SD: standard deviation

regimens against the wild-type virus were 1,042 (828-1,311) and 10,485 (7,228-15,209) U/ml, respectively and the anti-RBD IgG levels were 1,475 (1,267-1,716) and 2,683 (2,075-3,469) BAU/ml, respectively.

The median (IQR) anti-S IgA S/C ratios among subjects who received the short- and long-term CV/BNT regimens were 6.6 (4.9-9.0) and 9.0 (7.8-9.0), respectively. The GMT (95% CI) total anti-RBD Ig level against the wild-type virus among subjects who received the CV/CV regimen was 98 (83-116) U/ml, the anti-RBD IgG level was 128 (114-144) BAU/ml and the median (IQR) anti-S IgA S/C ratio was 0.9 (0.6-1.8). The GMT (95% CI) total anti-RBD Ig level against the wild-type virus among those who received the BNT/BNT regimen was 1963 (1378-2798) U/ml, the anti-RBD IgG level was 1,651 (1,182-2,306) BAU/ml, and the median (IQR) anti-S IgA S/C ratio was 5.6 (4.5-8.4).

The total anti-RBD Ig levels among those who received the short- and long-interval CV/BNT regimen were significantly higher (<0.001, <0.001) than those who received the CV/CV regimen, and those who received the BNT/BNT regimen, (0.019, <0.001), respectively (Fig 1a). The GMT anti-RBD IgG levels among those who received the short- and long-interval CV/BNT regimen were significantly higher (<0.001, <0.001) than those who received the CV/CV regimen, but not significantly different from those who received the BNT/BNT regimen (0.814 and 0.774), respectively (Fig 1b). Subjects who received the long-interval CV/BNT regimen had a significantly higher total anti-RBD Ig level (<0.001) and anti-RBD IgG level (p = 0.023) than those who received the short-interval CV/BNT regimen.

Forty-three percent of subjects who received the CV/CV regimen had an elevated anti-S1 IgA level while 100% of subjects who had the CV/BNT and BNT/BNT regimens did (Fig 1c).

Neutralizing activity

The median (IQR) percent inhibition (neutralizing activity) found among subjects vaccinated with the short-interval CV/BNT regimen against the wild-type, Alpha, Beta, Delta and Omicron variants were: 96(93-97), 85 (79-89), 77 (69-83), 93 (89-95), and 27 (22-35), respectively (Figs 2a-e). The median (IQR) percent inhibition (neutralizing activity) found among subjects vaccinated with the

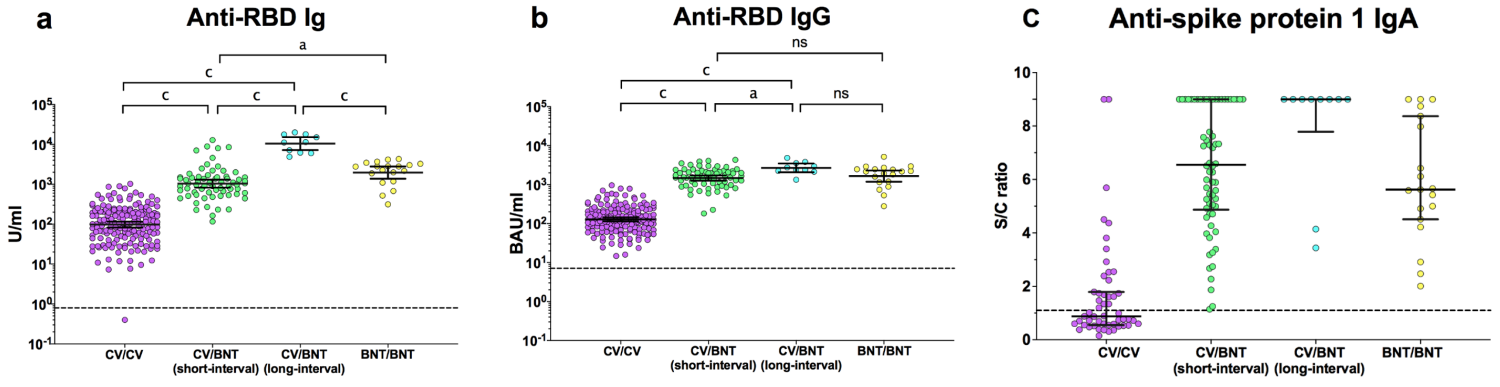

Fig 1 - SARS-CoV-2 specific binding antibodies

a: Total immunoglobulin specific to the RBD (anti-RBD Ig); b: Anti-RBD IgG; c: Anti-spike protein 1 IgA (anti-S1 IgA) 21-35 days after 2nd dose of the vaccine

Data points are the reciprocals of the individual. Lines indicate geometric means and bars indicate 95% confidence intervals for anti-RBD Ig and anti-RBD IgG, and median and interquartile ranges for anti-S1 IgA. Dotted lines denote the manufacturer's suggested positive cut-off levels.

[a]$p \leq 0.05$; [b]$p \leq 0.01$; [c]$p \leq 0.001$

BAU/ml: Binding antibody units per milliliter; BNT: mRNA BNT162b2 COVID-19 vaccine (Pfizer-BioNTech Inc, New York City, NY); CV: CoronaVac vaccine (Sinovac Life Sciences Co Ltd, Beijing, China); Ig: Immunoglobulin; IgA: Immunoglobulin A, IgG: Immunoglobulin G; ml: milliliter; ns: not significant; RBD: receptor-binding domain; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; S/C ratio: a sample-to-calibrator relative light unit index; U/ml: units per milliliter

long-interval CV/BNT regimen against the wild-type, Alpha, Beta, Delta and Omicron variants were: 97 (97-98), 98 (97-98), 95 (94-96), 97 (97-98), and 54 (38-84), respectively (Figs 2a-e). The median (IQR) percent inhibition (neutralizing activity) found among subjects vaccinated with the CV/CV regimen against the wild-type, Alpha, Beta, Delta and Omicron variants were: 67 (49-79), 42 (29-58), 35 (21-47), 49 (36-63), and 11 (9-15), respectively (Figs 2a-e). The median (IQR) percent inhibition (neutralizing activity) found among subjects vaccinated with the BNT/BNT regimen against the wild-type, Alpha, Beta, Delta and Omicron variants were: 97 (96-98, 94 (88-95), 89 (80-91), 97 (95-98), and 33 (27-42), respectively (Figs 2a-e). We found the median percent inhibition (neutralizing activity) against the Omicron variant found among subjects vaccinated with the short- and long-interval CV/BNT regimens were significantly higher than among subjects vaccinated with the CV/CV regimen ($p<0.001$, $p<0.001$, respectively) but not significantly different than among those vaccinated with the BNT/BNT regimen ($p = 0.416$, $p = 0.492$), respectively. Subjects vaccinated with the long-interval CV/BNT regimen had significantly higher

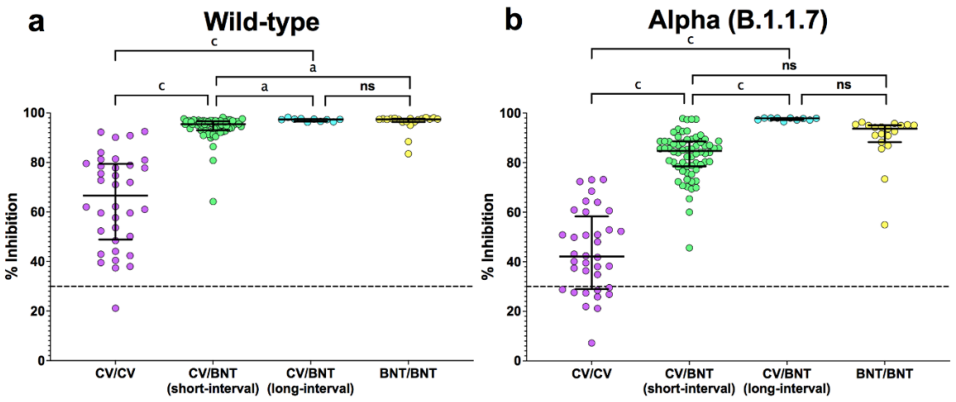

Fig 2 - Neutralizing activities against SARS-CoV2 wild-type and variants: (a) wild-type (b) Alpha (B.1.1.7), (c) Beta (B.1.351), (d) Delta (B.1.617.2) and (e) Omicron BA.4/5 among study subjects 21-35 days after second vaccine dose

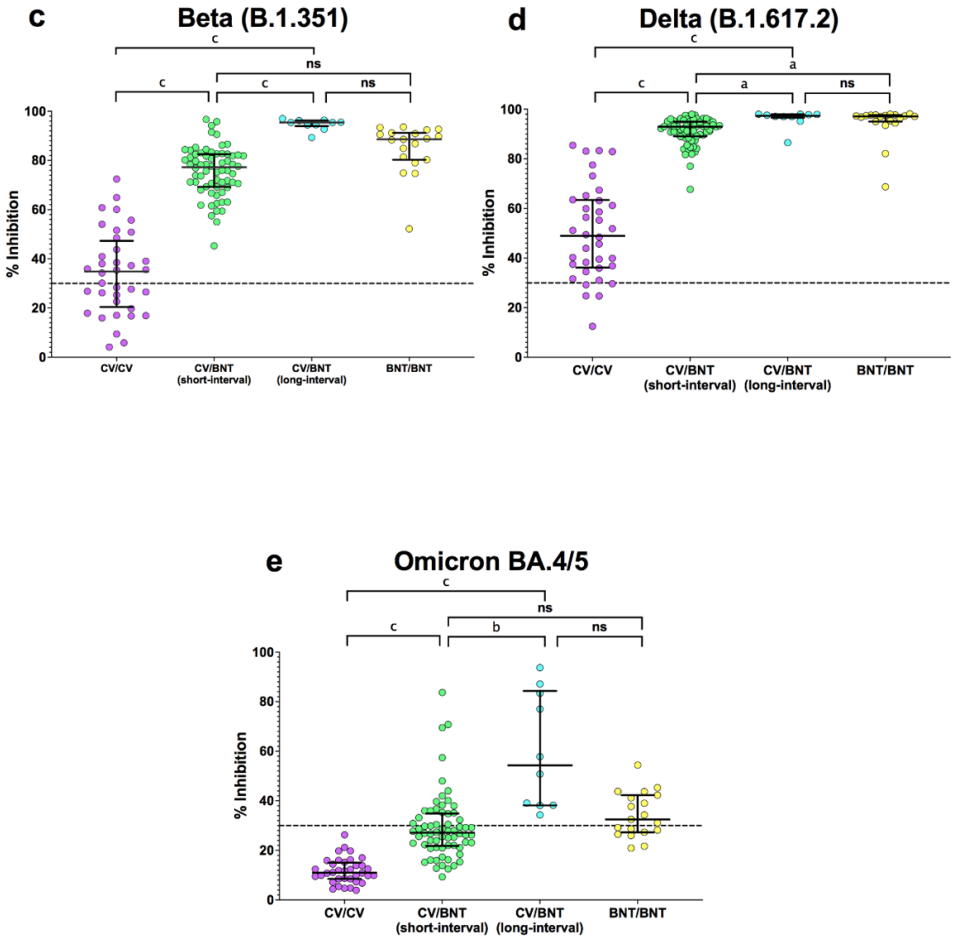

Fig 2 - (cont)

Neutralizing activities against SARS-CoV2 wild-type and variants: (a) wild-type (b) Alpha (B.1.1.7), (c) Beta (B.1.351), (d) Delta (B.1.617.2) and (e) Omicron BA.4/5 among study subjects 21-35 days after second vaccine dose

The data points are the reciprocals of the individual. Lines indicate medians and I-bars indicate interquartile ranges. Dotted lines denote the manufacturer's suggested positive cut-off levels.

[a]$p \leq 0.05$; [b]$p \leq 0.01$; [c]$p \leq 0.001$

BNT: mRNA BNT162b2 COVID-19 vaccine (Pfizer-BioNTech Inc, New York City, NY); CV: CoronaVac vaccine (Sinovac Life Sciences Co Ltd, Beijing, China); SARS-CoV-2: severe acute respiratory syndrome coronavirus 2

neutralizing activity the subjects vaccinated with the short-interval regimen against the wild (p = 0.045), Alpha (p<0.001), Beta (p<0.001), Delta (p = 0.026) and Omicron (p= 0.004) variants.

DISCUSSION

In our study the GMT of the total anti-RBD Ig and anti-RBD IgG levels among subjects vaccinated with the short- and long-interval CV/BNT regimens were higher than with the CV/CV regimen after adjusting for age and sex, similar to the results of previous studies showing: the CV/ChAdOx1-S regimen elicited a greater immune response than the CV/CV regimen (Wanlapakorn *et al*, 2022a), the ChAdOx1-S/BNT regimen elicited a greater immune response than the ChAdOx1-S/ ChAdOx1-S regimen (Barros-Martins *et al*, 2021; Borobia *et al*, 2021), the ChAdOx1-S/mRNA1273 regimen elicited a greater immune response than the ChAdOx1-S/ ChAdOx1-S regimen (Normark *et al*, 2021) and the CV/AD5-nCOV regimen elicited a greater immune response than the CV/CV regimen (Li *et al*, 2022). Previous studies have hypothesized the improved immunogenicity associated with the inactivated/viral-vectored or inactivated/mRNA COVID-19 heterologous regimens may result from memory responses targeted to the spike protein, rather than the whole virus, since the inactivated virus vaccine contains more non-neutralizing viral epitopes than spike protein levels, thus eliciting a lower immune response (Li *et al*, 2022). The greater immunogenicity seen with the viral-vectored/mRNA regimen compared to two-dose viral-vectored vaccine may be attributable to a potential issue of anti-viral vector immunity (Kardani *et al*, 2016). A heterologous regimen has the potential to generate a stronger cellular immune response, which is associated with a higher and more specific antibody response (Kardani *et al*, 2016).

In our study, subjects vaccinated with the long-interval CV/BNT regimen had higher anti-RBD immunoglobulin, anti-RBD IgG and neutralizing activities against the wild type and other variants tested than the short-interval regimen, similar to the results of other studies (Shaw *et al*, 2022; Parry *et al*, 2022). A previous study among Thai adolescents reported after receiving the CV/BNT regimen the GMT of anti-S-RBD IgG against the wild type variant was

significantly higher among those who received their two-dose vaccine regimen 6 weeks apart than those who received it 3 weeks apart (Puthanakit *et al*, 2022). A longer interval between the first a second doses is usually associated with a greater humoral immune response since the affinity maturation of memory B cells induced by vaccination may take several months (Sallusto *et al*, 2010). A long-interval heterologous regimen has not been publicly recommended since the first dose of the regimen is insufficient to stimulate an adequate immune response leaving the individual at risk until they receive the second dose (Jara *et al*, 2021), which is not ideal early in a pandemic when a rapid immune response is needed.

In our study, subjects vaccinated with the CV/BNT regimen had greater NA against the wild type and other tested variants than those vaccinated with the CV/CV regimen. A previous study reported finding a significant positive association between anti-RBD IgG levels and neutralization titers, suggesting anti-RBD IgG levels may serve as a correlate of neutralization (Lustig *et al*, 2021). NA has been reported to be significantly positively associated with immune protection (Khoury *et al*, 2021). These results suggest the CV/BNT regimen could offer better protection against the wild type and other tested variants than the CV/CV regimen.

IgA antibodies are often detectable before the appearance of SARS-CoV-2-specific IgG, suggesting IgA antibodies might be important for early virus neutralization (Bureerug *et al*, 2023). In our study, those who received the CV/BNT and BNT/BNT regimens had greater anti-S1 IgA S/C ratios than those who received the CV/CV regimen. However, it is unclear what clinical benefit this has in vivo but it could mean better NA against SARS-CoV-2 (Sterlin *et al*, 2021; Klingler *et al*, 2021; Wang *et al*, 2021).

Our study had several limitations, the first being that the great majority of study subjects were females. This could not adequately be adjusted for, indicating these results may not adequately reflect the effect of sex on the results. A second limitation was the small study sample, which may not detect factors with a minor but significant impact on the results. A third limitation was the threshold effect of cut off levels which may vary by factors not controlled for affecting our results.

In summary, the CV/BNT regimen

provided mean anti-RBD IgG levels higher than the two-dose CV regimen. We conclude the heterologous CV/BNT combination regimen may be a reasonable alternative for initial vaccination. However, further studies regarding efficacy over time need to be conducted before this can be confirmed.

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CONFLICTS OF INTEREST DISCLOSURE

The authors declare no conflicts of interest.

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