Original Article

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Comparison of Antibody Responses in Older Adults Who Had Natural Infection vs. Those Who Received an mRNA Booster Following ChAdOx1 nCoV-19 Vaccination

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Abstract:

Objective: This study compared antibody responses in elderly patients who received 2 doses of Chimpanzee Adenovirus Oxford 1 – novel Coronavirus 2019 (ChAdOx1 nCOV-19) and later contracted coronavirus disease 2019 (COVID-19) (COVID-19 group; CVD) with those who remained uninfected but received an mRNA booster (COVID-19 and vaccine group; CVV).

Material and Methods: A study was conducted during an outbreak at a nursing home between October and November 2021. Antibodies were tested at 12±2 weeks after recovery from COVID-19 or after the mRNA vaccine booster.

Results: Forty-three patients in the CVD group and 16 patients in the CVV group were enrolled. The levels of neutralizing antibodies against SARS-CoV-2 (% inhibition) were 97.9 (interquartile range (IQR) 97.3-98.2) and 96.8 (IQR 75.2-97.8); p-value=0.007 for wild-type SARS-CoV-2, 98 (IQR 97-98.5) and 88.3 (IQR 55.2-96.8); p-value<0.001 against B.1.1.7 (Alpha), 95.9 (IQR 90.2-97.7) and 79.1 (IQR 47-88.5); p-value<0.001 against B.1.351 (Beta), 98.1 (IQR 97.4-98.4) and 84 (IQR 37.3-96.6); p-value<0.001 against B.1.617.2 (Delta), and 28.2 (IQR 0-66) and 17.0 (IQR 1.5-36.9); p-value=0.388 against B.1.1.529 (Omicron), respectively.

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Conclusion: Hybrid immunity elicited higher neutralizing antibody levels than vaccination alone. However, for newer variants like Omicron, both hybrid immunity and vaccination alone resulted in low neutralizing antibody levels during the study period.

Keywords: ChAdOx1 nCOV-19 vector vaccine, COVID-19, mRNA vaccine, neutralizing antibodies, RBD-specific IgG antibodies

Introduction

In March 2020, the World Health Organization declared the coronavirus disease 2019 (COVID-19) a pandemic[1]. Since then, multiple vaccines with proven effectiveness have been deployed globally[2]. In Thailand, the vaccination effort began in 2021, with the introduction of inactivated virus vaccines (Sinovac-CoronaVac, Sinopharm), followed by the viral vector vaccine (ChAdOx1 nCoV-19) and mRNA vaccines (BNT162b2, Pfizer-BioNTech; mRNA-1273, Moderna-NIAID)[3]. While vaccines initially showed high efficacy against wild-type SARS-CoV-22-5, their performance against variants of concern (VOCs) has declined[4]. VOCs are defined as viral strains with genetic mutations that increase transmissibility or virulence, or reduce the effectiveness of public health measures, diagnostics, vaccines, or therapeutics[5]. At the time this study was conducted, the designated VOCs included: 1) B.1.1.7 (Alpha), 2) B.1.351 (Beta), 3) P.1 (Gamma), 4) B.1.617.2 (Delta), and 5) B.1.1.529 (Omicron), with the Delta variant being the predominant circulating strain. An early report from South Africa demonstrated a rapid decline in the efficacy of the mRNA vaccine (BNT162b2) against the Omicron variant compared to Delta, likely due to the increased number and altered locations of mutations in the spike protein, and a third (booster) dose of mRNA vaccines was therefore required[4].

Hybrid immunity, a combined immunity against SARS-CoV-2 obtained from vaccination and natural infection, regardless of the order, elicits both humoral and cell-mediated immune responses more effectively than either vaccination or natural infection alone[6-8]. For example, neutralizing antibodies against the Beta variant were up to 25 times higher than after vaccination alone and 100 times higher than after infection alone[7]. Memory B cells were also significantly more robust when vaccination followed infection[7]. This enhanced response is likely due to B cell diversity shaped by T cell help in the germinal center, particularly from T follicular helper and $CD4^+$ T cells[7]. Unlike vaccines, which target only the spike protein, natural infection induces broader T cell immunity, and hybrid immunity combines both, offering potent protection against VOCs[7,9,10]. A meta-analysis of 26 studies confirmed that hybrid immunity provides the strongest and most durable protection against Omicron and severe COVID-19[11].

In October 2021, a COVID-19 outbreak occurred in a nursing home in Chiang Mai, where most residents had received 2 doses of the ChAdOx1 nCoV-19 vaccine prior to the outbreak. Approximately two-thirds of the residents contracted COVID-19, while one-third remained uninfected. We therefore conducted this study to evaluate the antibody response, specifically receptor-binding domain (RBD)-specific IgG antibodies and neutralizing antibodies, among the elderly individuals who had received 2 doses of the ChAdOx1 nCoV-19 vaccine, and either developed COVID-19 during the outbreak (natural infection) or remained uninfected and later received an mRNA vaccine as a third dose (booster). We also assessed the antibody response against the following VOCs: 1) B.1.1.7 (Alpha), 2) B.1.351 (Beta), 3) P.1 (Gamma), 4) B.1.617.2 (Delta), and 5) B.1.1.529 (Omicron).

Material and Methods

This prospective cohort study was conducted during the outbreak between October 25 and November 5, 2021, in a nursing home under the medical care of Maharaj Nakorn Chiang Mai Hospital, Faculty of Medicine, Chiang Mai University. Participants were elderly individuals who met the eligibility criteria. The inclusion criteria were age over 60 years, had received 2 doses of the ChAdOx1 nCOV-19 vaccine as a primary series vaccine, and provided informed consent. They were divided into 2 groups: those confirmed to have SARS-CoV-2 infection by a real-time reverse transcriptase-polymerase chain reaction (RT-PCR) (CVD group) and those uninfected who received mRNA-1273 as a booster (CVV group). Blood samples were collected 12±2 weeks after recovery (CVD group) and at 4±1 weeks and 12±2 weeks post-booster (CVV group). Exclusion criteria included elderly individuals who had never received any SARS-CoV-2 vaccines prior to the outbreak, had received a vaccine other than ChAdOx1 nCoV-19 as their primary series, or had already received a booster dose of any SARS-CoV-2 vaccine before the outbreak; and in the CVV group, had received a lived attenuated vaccine within 28 days before receiving the mRNA booster.

Laboratory assays

RBD-specific IgG antibodies were measured using the SARS-CoV-2 IgG II Quant assay (Abbott Laboratories Inc., Illinois, USA)[12]. The quantitative antibodies are presented as arbitrary units (AU)/mL and then converted to binding antibody units (BAU)/mL by multiplying by 0.142 per WHO guidelines. The cut-off level for a positive result was ≥50 AU/mL or 7.1 BAU/mL[13].

Neutralizing antibodies were measured using an in-house SARS-CoV-2 surrogate virus neutralization test (sVNT) to assess antibody levels against wild-type (WT) SARS-CoV-2 and VOCs, including B.1.1.7 (Alpha), B.1.351 (Beta), B.1.617.2 (Delta), and B.1.1.529 (Omicron) variants, as previously described[14]. In brief, the microplate wells were coated with angiotensin-converting enzyme 2 (ACE-2) (GenScript, Piscataway, New Jersey, USA) in a bicarbonate buffer (pH 9.6) and incubated overnight at 4°C. Plates were washed 4 times with phosphate buffer saline (PBS) containing 0.05% Tween-20, and 100 µL of 2% bovine serum albumin (PAA Laboratory, Pasching, Austria) was added to each well. This was followed by a 1-hour incubation at 37 °C, and supernatants were discarded. A mixture of sera pre-incubated with horseradish peroxidase (HRP)-conjugated RBD in a volume of 100 µL was then added to each well, and plates were incubated at 37°C for 30 min. Plates were washed 4 times, and 50 µL of 3,3′,5,5′-tetramethylbenzidine (TMB) substrate solution (Life Technologies, Frederick, Maryland, USA) was added. Next, the plates were incubated at room temperature for 30 min. The reaction was then stopped by the addition of 0.2 M sulfuric acid. Plates were read spectrophotometrically at 450 nm on a CLARIOstar® microplate reader (Ortenberg, Germany). The cut-off level of seroconversion was 30% inhibition.

Descriptive data are presented as number (%), mean (standard deviaition (S.D.)), or median (interquartile range (IQR)). Student's t-tests and Wilcoxon signed-rank tests were used for group comparisons. Analyses were performed using Stata 10.0, with figures generated in R 4.1.2. A p-value<0.05 was considered significant. All statistical analyses were performed using Stata statistical software version 10.0 (Stata Corporation, College Station, TX, 2007), with figures generated using R version 4.1.2 (R Foundation for Statistical Computing, Vienna, Austria). A two-sided test was used to indicate statistical significance at a p-value<0.05.

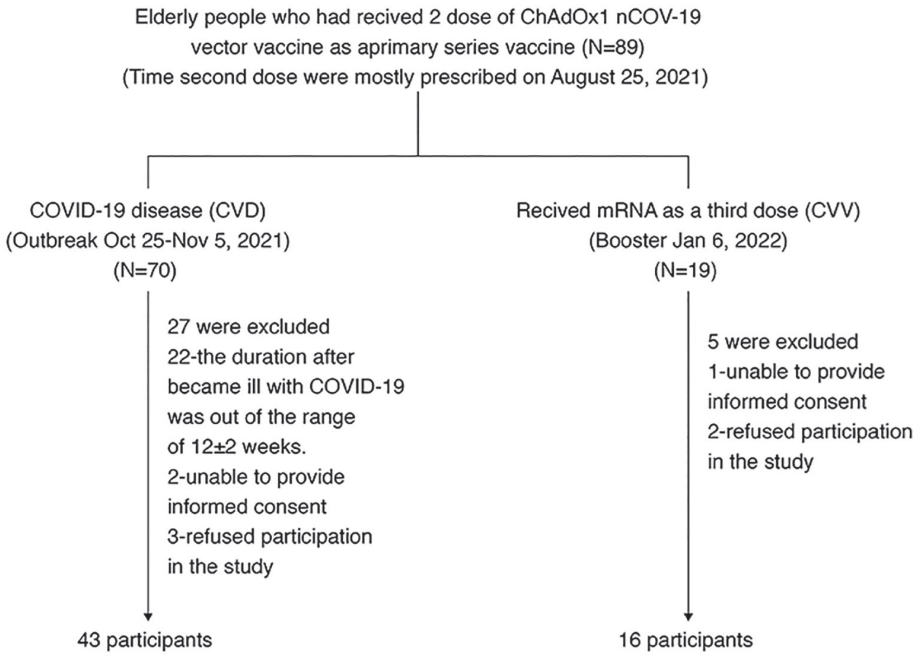
Results

Of the 89 residents who received 2 doses of ChAdOx1 nCoV-19, 70 contracted COVID-19, and 19 received an mRNA booster. Fifty-nine met the inclusion criteria (Figure 1): 43 in the CVD group and 16 in the CVV

group. The median ages (IQR) were 77 (71, 83) years in the CVD group and 81 (76, 83.5) years in the CVV group (p-value 0.186), with the CVD group having a higher proportion of males (62.8%) compared to none in the CVV group (Table 1). The median time from the second vaccine dose to infection in the CVD group was 121 days (IQR 119-127), while it was 134 days (IQR 134-134) to the booster in the CVV group (p-value<0.001).

The median time from the second dose of the primary series to infection in the CVD group was 121 days (IQR 119–127), whereas the median time from the second dose to receiving the booster in the CVV group was 134 days (IQR 134–134) (p-value<0.001).

At 12±2 weeks after recovery from COVID-19 in the CVD group and after mRNA vaccination in the CVV group, sVNT levels against SARS-CoV-2 (% inhibition) were significantly higher in the CVD group across most variants. For wild-type SARS-CoV-2, the median inhibition was 97.9% (IQR 97.3–98.2) in the CVD group and 96.8% (IQR 75.2–97.8) in the CVV group (p-value=0.007). Against the B.1.1.7 (Alpha) variant, inhibition levels were 98% (IQR 97–98.5) and 88.3% (IQR 55.2–96.8), respectively (p-value<0.001). For B.1.351 (Beta), levels were 95.9% (IQR 90.2–97.7) in the CVD group and 79.1% (IQR 47–88.5) in the CVV group (p-value<0.001). For B.1.617.2 (Delta), inhibition levels were 98.1% (IQR 97.4–98.4) and 84% (IQR 37.3–96.6), respectively (p-value<0.001). In contrast, inhibition against the B.1.1.529 (Omicron) variant was low in both groups, at 28.2% (IQR 0–66) in the CVD group and 17% (IQR 1.5–36.9) in the CVV group, with no statistically significant difference (p-value=0.388). These findings are shown in Table 2 and Figure 2.



COVID-19 group (CVD) refers to elderly individuals who had received 2 doses of the Chimpanzee Adenovirus Oxford 1 – novel Coronavirus 2019 (ChAdOx1 nCOV-19) and later contracted COVID-19, COVID-19 and vaccine group (CVV) refers to elderly individuals who had received 2 doses of the ChAdOx1 nCOV-19 and remained uninfected but received an mRNA booster.

Figure 1 Study flow of participants

Table 1 Baseline characteristics of the participants in the CVD and CVV groups

Characteristics	CVD (n=43)	CVV (n=16)	Total (N=59)	p-value
Median age (IQR)	77 (71, 83)	81 (76, 83.5)	77 (74, 83)	0.186
Female n (%)	16 (37.2)	16 (100)	32 (54.2)	<0.001
Underlying diseases (n, %)				
Diabetes mellitus	4 (9.3)	5 (31.3)	9 (15.3)	0.052
Hypertension	25 (58.1)	11 (68.8)	36 (61)	0.458
Dyslipidemia	18 (41.9)	10 (62.5)	28 (47.5)	0.158
Chronic kidney disease	5 (11.6)	2 (12.5)	7 (11.9)	>0.99
Heart diseases	2 (4.7)	1 (6.3)	3 (5.1)	>0.99
Lung diseases	7 (16.3)	1 (6.3)	8 (13.6)	0.427
Neurological diseases	7 (16.3)	6 (37.5)	13 (22)	0.154
Liver diseases	1 (2.3)	–	1 (1.7)	>0.99
Other diseases	21 (48.8)	4 (25)	25 (42.4)	0.100

COVID-19 group (CVD) refers to elderly patients who became ill with COVID-19 after receiving the primary vaccine series, COVID-19 and vaccine group (CVV) refers to elderly people who did not become ill with COVID-19 but received an mRNA vaccine as a third dose.

Table 2 Antibody levels against SARS-CoV-2 between the participants in the CVD and CVV groups

Antibodies	CVD (n=43)	CVV (n=16)	p-value
Geometric mean titers of RBD-specific IgG, BAU/mL (95% CI)			
At 4±1 weeks after the third dose of vaccine	N/A	1440.5 (906.8, 2288.4)	
At 12±2 weeks after became ill with COVID-19 in the CVV group and after the third dose of vaccine in the CVV group	1632.1 (1109.1, 2401.7)	450.4 (256.1, 792.2)	0.001
Median (IQR) % inhibition of neutralizing antibodies against SARS-CoV-2 at 12±2 weeks			
Wild-type (Wuhan)	97.9 (97.3, 98.2)	96.8 (75.2, 97.8)	0.007
B.1.1.7 (Alpha)	98.0 (97.0, 98.5)	88.3 (55.2, 96.8)	<0.001
B.1.351 (Beta)	95.9 (90.2, 97.7)	79.1 (47.0, 88.5)	<0.001
B.1.617.2 (Delta)	98.1 (97.4, 98.4)	84.0 (37.3, 96.6)	<0.001
B.1.1.529 (Omicron)	28.2 (0, 66.0)	17.0 (1.50, 36.9)	0.388
Seroconversion rate (n, %)			
Wild-type (Wuhan)	40 (93)	15 (93.8)	>0.99
B.1.1.7 (Alpha)	40 (93)	14 (87.5)	0.606
B.1.351 (Beta)	40 (93)	15 (93.8)	>0.99
B.1.617.2 (Delta)	40 (93)	13 (81.3)	0.330
B.1.1.529 (Omicron)	20 (46.5)	4 (25)	0.135

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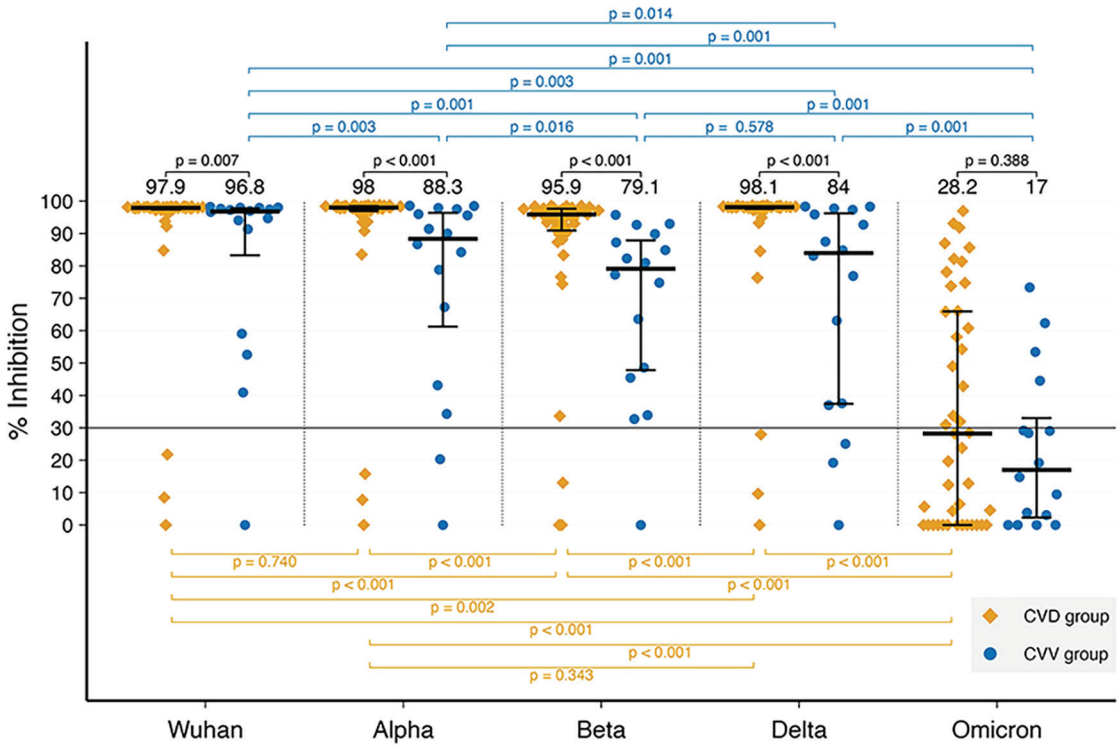

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Figure 2 Neutralizing antibodies against wild-type-SARS-CoV-2 and variants of concern

The geometric mean titer (GMT) of RBD-specific IgG antibodies was 1,632.1 BAU/mL (95% CI, 1,109.1–2,401.7) in the CVD group and 450.4 BAU/mL (95% CI, 256.1–792.2) in the CVV group, as shown in Table 2. In the CVV group, the GMT declined from 1,440.5 BAU/mL (IQR 906.8–2,288.4) at 4±1 weeks to 450.4 BAU/mL (IQR 256.1–792.2) at 12±2 weeks post-booster (p-value=0.001), as shown in Figure 3. There were no differences in seroconversion rates between the groups (Table 2).

Discussion

This study highlights and confirms the superior immune response generated by hybrid immunity compared to vaccination alone[6,7,10,11]. The CVD group demonstrated higher neutralizing antibody levels across multiple variants, consistent with previous findings that hybrid immunity offers broader and more durable protection[7,10]. Baseline characteristics showed that all participants in the CVV group and 37.2% in the CVD group were female. Substantial evidence indicates that females often mount more robust immune responses to vaccines, leading to higher antibody production and greater vaccine efficacy[15,16]. However, the CVV group, which comprised only females, demonstrated lower antibody levels than the CVD group. This suggests that prior infection may have had a stronger impact on immune response than sex-based immunologic differences in this cohort. Furthermore, although the difference in diabetes mellitus (DM) prevalence between the groups

was not statistically significant (p-value=0.052), the higher proportion of individuals with diabetes in the CVV group may have contributed to the lower antibody responses observed. People with DM are known to exhibit impaired immune responses to vaccination[17,18], which may partially explain the trend toward weaker immunogenicity in the CVV group. However, DM was not associated with antibody levels in this study. Notably, for the Omicron variant, both the CVD and CVV groups showed markedly lower neutralizing activity with wider variability compared to other variants (Figure 2). The distributions overlapped substantially, and the difference between groups was not statistically significant (p-value=0.388). This pattern likely reflects the immune evasion capacity of Omicron, likely due to its extensive spike protein mutations, and highlights the limited cross-neutralizing immunity provided by prior infection or ancestral strain vaccination[19-21]. Compared to the CVD group, antibody levels in the CVV group showed greater variability. A biphasic distribution was observed, with some individuals mounting strong responses while others had substantially lower titers. This heterogeneity may reflect individual differences in immune response to the booster vaccine, potentially influenced by immunosenescence[22], underlying comorbidities[18,23,24], or undetected prior infection.

As SARS-CoV-2 evolves, new variants like JN.1 exhibit increased immune escape potential[25]. Vaccines administered during the 2021 outbreak may no longer provide adequate protection. Waning immunity, along with the emergence of new variants, highlights the need for updated vaccine formulations and timely booster doses, particularly those tailored to circulating strains. This is especially true in the elderly, given the impact of immunosenescence, characterized by reduced B cell and T cell function, which contributes to weaker vaccine-induced

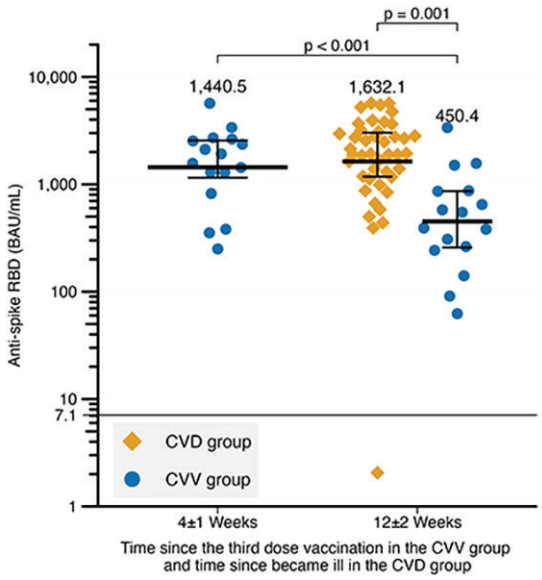

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Figure 3 Receptor-binding domain (RBD)-specific antibody against WT-SARS-CoV-2 between participants in the CVD and CVV groups

immune responses and more rapid waning of protection[22]. However, despite booster recommendations, as of March 2023, only 40.0% of the population had received a third dose, 10.0% a fourth dose, and just 1.5% a fifth dose[26]. This highlights the urgent need to improve booster vaccine acceptance, particularly to protect vulnerable populations.

This study has several limitations. First, the sample size was small, which necessitates cautious interpretation of the results. Second, anti-nucleocapsid antibodies were not measured in the CVV group, leaving asymptomatic or subclinical infections unconfirmed, though immune responses may be less pronounced in such cases[27]. This may have led to group misclassification and introduced potential bias in interpreting the findings. Third, the interval between the second vaccine dose and booster in the CVV group was 2 weeks longer than the recovery period in the CVD group, potentially influencing antibody responses, as longer intervals can enhance responses[28]. Fourth, RBD-specific antibodies were not measured at one month post-recovery in the CVD group, limiting comparisons of antibody decline between the groups. Lastly, cell-mediated immune responses were not assessed, although other studies have emphasized the role of cross-reactive T cell responses following infection[29].

Conclusion

In conclusion, hybrid immunity elicited higher antibody levels against SARS-CoV-2 variants, excluding Omicron, compared to vaccination alone. The observed waning of antibody levels after a booster dose underscores the importance of continued vaccination efforts with updated booster formulations tailored to emerging variants, particularly in elderly and vulnerable populations who are at risk of severe COVID-19.

Ethical considerations

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Research Ethics Committee, Number 5, Faculty of Medicine, Chiang Mai University (Approval No. 046/2022). Informed consent was obtained from all participants.

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Conflict of interest

All authors declare no conflict of interest.

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