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Anti-SARS-CoV-2 receptor binding domain antibodies after the second dose of Sinovac and AstraZeneca vaccination

Purpose: The Sinovac and AstraZeneca vaccines are the primary coronavirus disease 2019 vaccines in Indonesia. Antibody levels in vaccine-injected individuals will decline substantially over time, but data supporting the duration of such responses are limited. Therefore, this study aims to quantitatively evaluate antibody responses resulting from the completion of Sinovac and AstraZeneca administration in Indonesian adults.

Materials and Methods: Participants were divided into two groups based on their vaccine type. Both groups were then assessed on the anti-severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) receptor binding domain (anti-SRBD) concentrations. The anti-SRBD level was measured using Elecsys anti-SARS-CoV-2 S assay and analyzed every month until 3 months after the second vaccination.

Results: The results presented significant differences ($p=0.000$) in immunoglobulin G (IgG) titers among the vaccines' measurement duration, where all samples observed a decrease in IgG titers over time. The mean titer levels of anti-SRBD IgG in the group given Sinovac were high in the first month after vaccination and decreased by 55.7% in 3 months. AstraZeneca showed lesser immune response with a slower decline rate. Adverse effects following immunization (AEFI) showed that systemic reactions are the most reported in both vaccines, with a higher percentage in the second dose of AstraZeneca type vaccines.

Conclusion: Sinovac induced more significant titers of anti-SRBD IgG 1 month after the second dose but generated fewer AEFIs. In contrast, AstraZeneca generated more AEFIs, in mild to moderate severity, but provided lower levels of anti-SRBD IgG.

Keywords: COVID-19, Vaccination, Anti-SARS-CoV-2 RBD antibody, Sinovac, AstraZeneca

Introduction

In January 2021, Sinovac, an inactivated aluminum-adjuvant vaccine developed by Sinovac Life Science Company (Beijing, China) [1], was selected for the initial vaccination phase in Indonesia due to its immediate availability [2]. Following Sinovac, an adenovirus vector vaccine, AstraZeneca (AstraZeneca, Cambridge, UK), was also permitted to use in Indonesia after receiving Emergency Use Authorization from the World Health Organization (WHO). Both vaccines have been employed for the national coronavirus disease 2019 (COVID-19) vaccination campaign to achieve herd immunity.

A significant phase III trial conducted in Brazil showed that administering two doses of Sinovac with a 14-day interval resulted in a 51% efficacy in protecting against symptomatic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. While

the phase III trials conducted in the United States, Chile, and Peru indicated that AstraZeneca showed 76% efficacy in preventing symptomatic illness [3,4]. However, the current vaccines' ability to protect against initial infection and subsequent re-infection, as well as the duration of this protection in real-world scenarios, remains uncertain. Recent studies have emphasized the significance of elevated spike antibody titers in eliciting a defensive immune reaction against SARS-CoV-2 [5]. It is believed that antibodies targeting the spike protein of SARS-CoV-2, specifically the receptor binding domain (RBD), play a crucial role in priming the immune response and neutralizing the virus [6]. Anti-SARS-CoV-2 RBD antibodies attach to the angiotensin-converting enzyme 2-binding site on the viral RBD, thereby blocking the viral entry [7].

Therefore, we tried to examine the anti-SARS-CoV-2 RBD response 1 month after the second vaccine dose and whether this vaccination is effective in increasing people's immunity. This study also aims to know any decrease in the anti-SARS-CoV-2 RBD response after the first 3 months of observation. We also evaluate the incidence of adverse effects following immunization (AEFI).

Materials and Methods

After obtaining ethical clearance, a prospective longitudinal population-based study was conducted in Surabaya to investigate the effectiveness of SARS-CoV-2 anti-spike RBD immunoglobulin G (IgG) in individuals vaccinated with Sinovac and AstraZeneca vaccines. All participants who were administered two doses of Sinovac or AstraZeneca in a drive-thru vaccination program from June 2021 until September 2021 were included in the study. Participants who were immunocompromised or confirmed positive for COVID-19 during the study period were excluded from this study. Informed consents were obtained from all included participants.

Participants provided demographic data before the vaccination, including the use of medication, complementary medicines, multivitamins, herbal supplements, and alternative medicine. During each visit, their blood pressure (BP), the plasma level of random blood glucose (RBG), uric acid, total cholesterol, and hemoglobin were evaluated for correlation analysis. In a follow-up sheet, they were asked to record any AEFIs (temperature, local pain, high BP, redness, fatigue, arthralgia, or any symptoms) for 2 weeks after each dose.

Blood samples obtained at first, second, and third months after the second dose of vaccination were analyzed at the Labbio-

gen, Klampis Jaya, Surabaya, Indonesia. Total anti-S-RBD antibodies, including IgG levels were measured using the macroenzyme-linked immunosorbent assay automatic immunoassay analyzer Cobas e 411 module (Roche Diagnostic, Mannheim, Germany). A commercial kit (Elecsys anti-SARS-CoV-2 S-RBD, Roche) was used with the electro-chemiluminescent method. As per the manufacturer's specifications, the measurement range covered values from 0.40 to 250 U/mL with onboard 1:10 dilution and up to 2,500 U/mL with onboard 1:10 dilution. A cut-off index with a value lower than 0.80 U/mL was considered "negative" for anti-SARS-CoV-2 antibodies, while a value higher than 0.80 U/mL was considered positive. The U/mL was converted to binding antibody units per milliliter (BAU/mL) according to the WHO standard [8]. The correlation between U/mL and BAU (International Organization Management Service standard) is $1 \text{ U/mL} = 0.972 \text{ BAU/mL}$.

The data were presented using numbers and percentages, as well as medians with ranges, and means with standard deviations. To analyze the difference between groups in categorical variables, the chi-square test was employed. Moreover, baseline characteristics, encompassing potential confounders such as participants' age, gender, and the presence of any underlying health conditions, were adjusted using analysis of covariance with Bonferroni correction. Data were analyzed using Prism ver. 9.9 (GraphPad Software, San Diego, CA, USA) and IBM SPSS ver. 24.0 (IBM Corp., Armonk, NY, USA). A non-parametric independent median test was performed to compare the rate of decline of anti-spike IgG across the age groups in the Sinovac and AstraZeneca group of vaccination, as well as a multivariate analysis to determine the factor(s) which was correlates most with the anti-spike IgG levels.

Results

Out of the 73 adults over 18 years old who had received two doses of Sinovac between June 2021 and August 2021, up to 57 individuals gave their informed consent to participate in the study, and a final count of 50 participants were eligible for inclusion. Between July and September 2021, 50 of the 55 adults given two doses of AstraZeneca were eligible for inclusion and provided informed consent. Therefore, a total of 100 participants were evaluated, with 50 in each vaccine group, and their baseline data are presented in Table 1.

The median age of those given Sinovac, 46 years (interquartile range [IQR], 35–50 years), was significantly higher ($p=0.002$) than the 39 years (IQR, 27–44 years) recorded in the AstraZene-

Table 1. Demographics and baseline characteristics of two vaccinated groups of Indonesian adults

Demographic characteristics	Normal value	Sinovac vaccinated	AstraZeneca vaccinated	p-value
Age (yr)	-	46 (35–50)	39 (27–44)	0.002
Sex				0.081
Male	-	21	29	
Female	-	29	21	
Body mass index (kg/m ²) ^{a)}				0.392
Underweight	<18.5	0	0	
Normal	18.5–22.9	20	25	
Overweight	23.0–24.9	19	18	
Obese	≥25.0	12	20	
Comorbidities ^{b)}				0.564
Normal		47 (94)	49 (98)	
Hypertension		1 (2)	0	
Asthma		1 (2)	0	
Hyperthyroid		1 (2)	1 (2)	
Blood pressure (mm Hg)				0.338
Normal	SBP <120 and DBP <80	11 (22)	19 (38)	
Prehypertension	SBP 120–139 or DBP 80–89	29 (58)	22 (44)	
Stage 1 hypertension	SBP 140–159 or DBP 90–99	10 (20)	9 (18)	
Stage 2 hypertension	SBP ≥160 or DBP ≥100	0	0	
Random blood glucose (mg/dL)				0.694
Normal	≤200	50 (100)	50 (100)	
Diabetes	≥200	1 (2)	0	
Total cholesterol (mg/dL)				0.480
Normal	125–200	40 (80)	41 (82)	
Borderline high	200–239	10 (20)	9 (18)	
High	>239	0	0	
Uric acid (mg/dL)				0.972
Normal				
Female	2.3–6.6	25 (86.2)	21 (100)	
Male	3.6–8.5	20 (95.2)	28 (96.6)	
More than normal				
Female	>2.3–6.6	4 (13.8)	-	
Male	>3.6–8.5	1 (4.8)	1 (3.4)	
Hemoglobin (g/dL)				0.331
Less than normal				
Female	<12–16	1 (3.4)	1 (4.8)	
Male	<13–18	-	2 (6.9)	
Normal				
Female	12–16	28 (96.6)	18 (85.7)	
Male	13–18	21 (100)	15 (51.7)	
More than normal				
Female	>12–16	-	2 (9.5)	
Male	>13–18	-	12 (41.4)	

Values are presented as median (interquartile range), number, or number (%).

SBP, systolic blood pressure; DBP, diastolic blood pressure.

^{a)}The values are expressed as body mass index for Asian. ^{b)}Comorbidities listed here are defined as medical diagnoses included in medical history by the International Classification of Diseases-10 coding. These include, but are not limited to, those presented in the table.

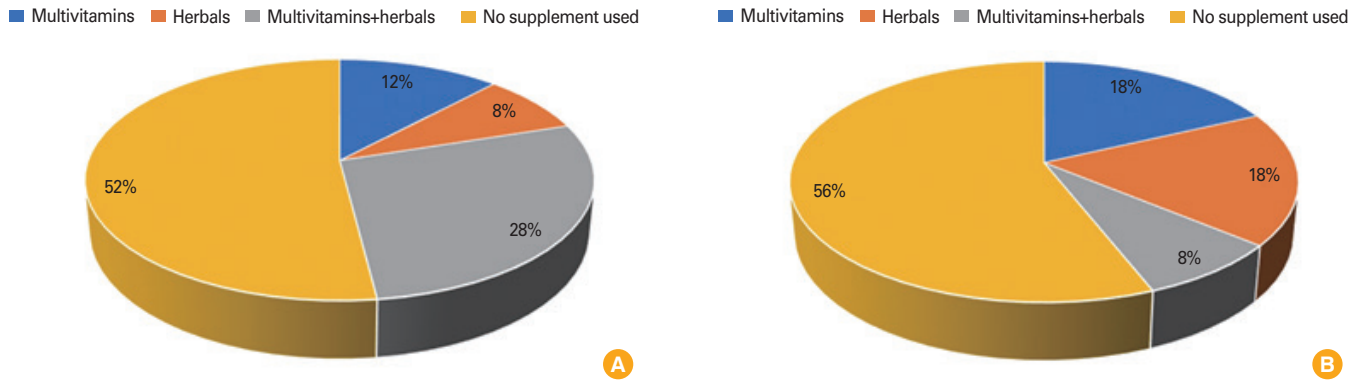


Fig. 1. Medication, complementary, and alternative medicine use, including multivitamin and herbal supplements of the Sinovac (A) and AstraZeneca (B) vaccinated group.

ca group. The vaccine groups showed a significant difference in mean age based on the Mann-Whitney test (43.20 [95% CI, 40.66–45.74] in the Sinovac group and 37.26 [95% CI, 34.53–39.99] in the AstraZeneca group) with a p-value of 0.001.

The use of medication, complementary medicines, multivitamins, herbal supplements, and alternative medicine was provided, as shown in Fig. 1. In the Sinovac group, it was observed that 40% of females aged 32–66 years used multivitamins and herbal supplements, while the other 40% of females and 66.7% of males did not. In the AstraZeneca group, 76.7% of males had no supplement, while 38.1% of females used herbs, 28.6% did not, 23.8% had multivitamins only, and 9.5% consumed both. The most frequently used herbal supplement in all groups was ginger, curcuma, turmeric, and honey.

The main focus of this study was to assess the percentage and magnitude of SARS-CoV-2 anti-spike RBD IgG antibody responses following vaccination in different age groups over time. The data revealed that the Sinovac vaccinated group exhibited a notably high titer level during the initial period (0–30 days) post-vaccination. Fig. 2 demonstrates the mean titers' level for each vaccine plotted against the predefined intervals post-vaccination. The anti-spike RBD IgG level's geometric mean after the second dose of Sinovac was 3,091 BAU/mL, 2,180 BAU/mL, and 1,370 BAU/mL in 1, 2, and 3 months, respectively. In the AstraZeneca group, the geometric mean was 2,034 BAU/mL, 1,553 BAU/mL, and 1,151 BAU/mL in 1, 2, and 3 months, respectively.

The decline rate of anti-spike IgG in the Sinovac vaccinated group was approximately 70% from 1 to 3 months post-vaccination, while AstraZeneca showed 75%. No-parametric independent sample median test was performed to compare the decline rate of the antibody across the age groups, and no significant difference was found in the decline rate over time between age groups ($p=0.818$) for both types of vaccines. How-

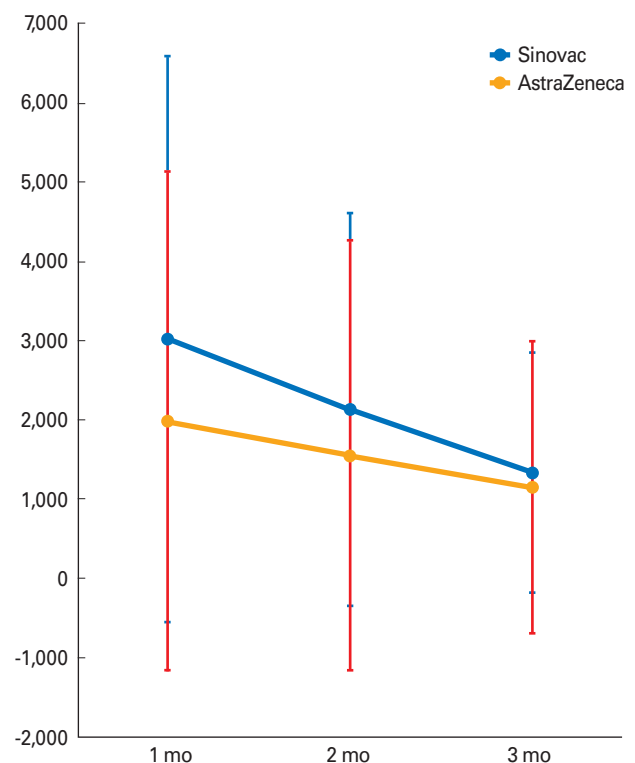


Fig. 2. Three-month observation of severe acute respiratory syndrome coronavirus 2 receptor binding domain antibodies after the second dose of Sinovac and AstraZeneca vaccination with error bar (=standard deviation).

ever, anti-spike IgG in each measurement time for both vaccines had significant differences ($p=0.000$), and the decline rate for the AstraZeneca group was slower.

Multivariate analysis was performed to determine the interaction between clinical conditions with the magnitude of SARS-CoV-2 anti-spike RBD IgG antibody (SRBD) post-vaccination responses over time. There were weak correlations, namely $r=0.404$, 0.298 , and 0.170 , and no significant differ-

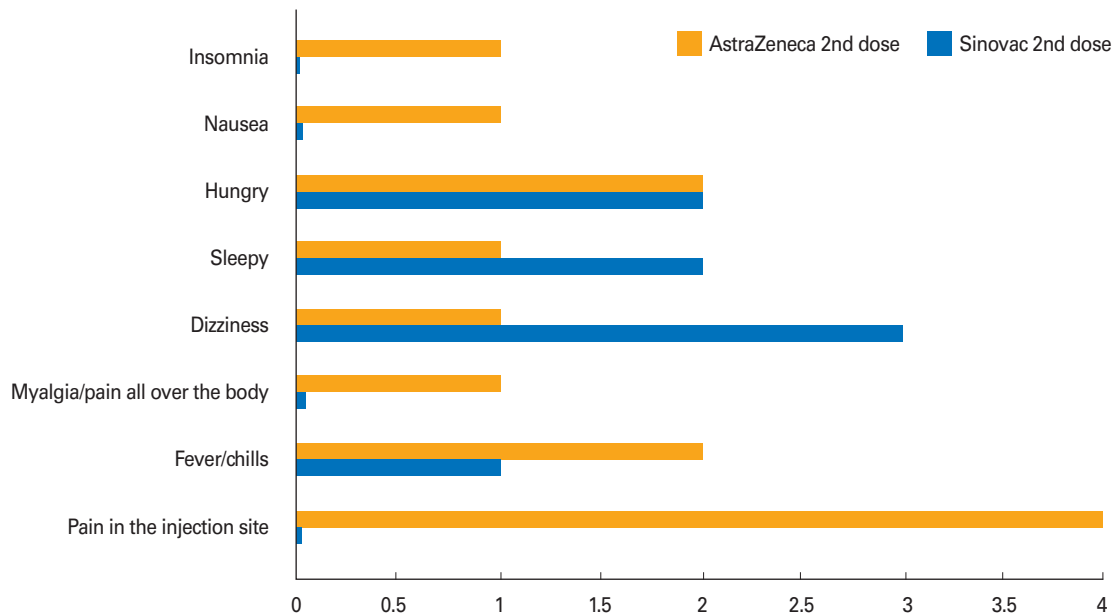


Fig. 3. Adverse effects following immunization of Sinovac and AstraZeneca 2nd dose vaccinated Indonesian adults.

ence, i.e., $p=0.594$, 0.888 , and 0.952 , respectively, between the first, second, and third SRBD measurements to the oxygen saturation (SpO_2), BP, body mass index (BMI), RBG, cholesterol, and uric acid level, as well as the body temperature. Despite its weak correlation, RBG was the most inversely correlated factor with the SRBD levels ($r=-0.493$).

It was identified that local AEFI showed a total of three reports (6%) in the first dose vaccination and none in the second Sinovac dose. Meanwhile, systemic reactions were higher in the first dose of Sinovac (30%) and lowered in the second dose (16%). As shown in Fig. 3, the AstraZeneca group showed less but a higher percentage of systemic reactions on the second dose (20%). The most systemic expected response for both vaccines was fever/chills. No allergic reactions were recorded from all vaccines type.

Discussion

The titers of anti-SRBD IgG were one of the methods to detect antibodies against the S-protein of the SARS-CoV-2 virus [9]. Anti-SRBD IgG binds to the RBD of SARS-CoV-2, possibly inhibiting viral attachment and preventing entry into the host cell, thereby impeding infection [10]. This testing strongly correlates with neutralizing antibodies, i.e., the gold standard of functional antibodies or immune response to vaccination. Utilizing this testing can aid in monitoring patients' responses after vaccination [11,12]. In this prospective observational

study, antibody titers were determined and compared until 3 months after the doses of the Sinovac and AstraZeneca were completed. The relationship of the host factor to the response was also investigated.

Antibody titers in the Sinovac group were discovered to be higher than in AstraZeneca 1 to 3 months after completing vaccination. The mean anti-SRBD IgG titers in the Sinovac group were the highest 1 month after vaccination, and then it began to drop after 90 days. Data showed that Sinovac titers reduced to the same level as the AstraZeneca at 90 days post-vaccination. Angkasekwinai et al. [13] found that Sinovac induced lower anti-SRBD IgG than AstraZeneca in Thailand's healthcare workers with geometric means of 164.4 BAU/mL and 278.5 BAU/mL. In this current study, no significant correlation was observed between gender or age and the antibody titers. However, Foddiss et al. [14] reported a significant correlation between these parameters. Other studies stated significantly higher anti-SRBD IgG in females and younger participants [15,16].

The results also showed a notable decline in the average antibody response after 3 months of vaccination for both vaccines. The rate of reduction of antibody levels was faster in the group vaccinated with Sinovac than in the group immunized with AstraZeneca. AstraZeneca showed a lower immune response but a slower decline rate. Favresse et al. [17] reported significant antibody titers' decline at 3 months compared to the peak response at 4 weeks post-vaccination with BNT162b2.

Barin et al. [18] found that the AstraZeneca group experienced a lower decline over time than Sinovac but with higher overall antibody titers. The current results were contrary to Wanlapakorn et al. [19], which discovered a two-dose Sinovac regimen elicited a more robust immune response when compared to the two-dose AstraZeneca regimen, contrary to their observation of a lower but acceptable immune response with Sinovac.

The decline rate over time did not differ significantly between the two age groups for each vaccine type. Previous studies have indicated that aging can lead to a decrease in cellular and humoral immunity with age, impacting vaccination responses [20,21]. Santi et al. [12] stated a significant difference in antibody titers after Sinovac vaccination based on age, with higher titers observed in the 26–39 age group. On the other hand, the neutralizing antibody titer of AstraZeneca did not differ significantly between the vaccinated population aged 18 to 55 years and those over 55 years [22]. Although females are reported to induce higher IgG titers, this current study did not find a significant difference. Moreover, observations from previous clinical trials confirmed that gender is not associated with cellular or antibody responses to the AstraZeneca vaccine [23]. Similarly, Lee et al. [24] also discovered that age and gender do not have an association with the antibody response to AstraZeneca.

In the two vaccines, there was a lack of association differences in clinical laboratory measurements after vaccination between antibody response and the host's clinical conditions, including SpO₂, BP, BMI, RBG, total cholesterol, and uric acid level, as well as the body temperature. According to earlier studies, COVID-19 antibody titers were higher in the underweight and normal-weight groups, which could be attributed to either infection or vaccination with a messenger RNA vaccine [25]. The variations in these findings could result from the different types of antibodies. Although no direct host factor differences were observed in the vaccine response, a stronger negative association of high blood glucose to antibody titers was detected. The data obtained further indirectly supported Islam et al. [26] that individuals with hyperglycemia exhibited lower spike IgG titers compared to their normoglycemic counterparts following the administration of two doses of the BNT162b2 vaccine. Moreover, the reduced production of neutralizing antibodies in diabetes indicates a weakened humoral immune response associated with [27]. A high amount of glucose-related signaling pathways ultimately leads to pro-inflammatory cytokine production and may initiate an impaired immune system function [28]. Zhang et al [3] observed an im-

paired immune system correlation with elevated fasting blood glucose in the presence of the SARS-CoV-2 omicron variant.

Supplements such as vitamins and herbs are beneficial for human health and play an essential role in boosting the immune system, which functions to combat COVID-19 [29]. In this study, no correlation of supplemental effect was detected on the antibody titers. Similarly, Ozgocer et al. [30] reported that the use of multivitamins or herbal therapies did not affect antibody response. The relationship between vitamins C, D, and herbs to IgG titers is unknown. Evidence supports that vitamin D has the potential to reduce the risk of COVID-19 infection by regulating the macrophage host defense system. This regulation leads to a decrease in viral replication rate and lowers the likelihood of virus-induced cytokine storms, particularly in individuals with obesity or chronic illnesses [31]. This also favors the induction of the T regulatory cells, thereby inhibiting inflammatory processes [32]. Evidence-based studies of herbal therapy against SARS-CoV-2 infection are still lacking. Ongoing clinical trials are being conducted to assess the impact of food supplements on COVID-19 prevention and treatment approaches [33].

This study showed that the local AEFI was higher in AstraZeneca than Sinovac vaccinated group after receiving the first or second dose. The local reaction occurred with a median duration of 5 hours in the two doses of AstraZeneca. The pain at the injection site was also evaluated with Visual Pain Score, and the AstraZeneca group was noted to complain at a median of 2 scales. Meanwhile, the Sinovac group complained at a median of 1 scale and showed a higher percentage of pain but mild in severity. Headache was the most reported AEFI in the second dose post-vaccination. Only 10 participants reported systemic reactions following the second dose of AstraZeneca, but the responses were more varied and moderate. Any drugs did not induce sleepiness before and after receiving vaccines.

Interestingly, the systemic reactions lasted longer in the AstraZeneca group than in Sinovac with a median duration of 24 hours. Most of the participants with AEFI used antipyretics such as acetaminophen. Systemic reactions, namely sleepiness and ear block, were reported in the Sinovac group.

AEFIs reactions to vaccines are suggested to be related to higher antibody levels [34,35] and they proved the relationship in the Sinovac group. Park et al. [36] showed an association between antibody response and systemic AEFIs after the first vaccine dose but not local reactions. This current study found no relationship between AEFIs, age, and gender. Meanwhile, Angkasekwina et al. [13] that compared the two vac-

cines in Thailand, reported significantly higher systemic AEFIs in the AstraZeneca group containing females younger than 30 years old.

This study was one of the first to show the difference between the antibody levels induced by Sinovac and AstraZeneca in Indonesia. In addition, it provided real-life data because a population with and without comorbidities was evaluated. However, there were several notable limitations; first, the study team failed to use anti-SRBD IgG before vaccinating participants with prior history of COVID-19, and no observation about the disease was conducted afterward. Second, the sample size was small and did not represent the general population. Third, more data are required for the elderly and younger ones.

In conclusion, Sinovac induced a high level of anti-SRBD IgG 1 month after the second dose compared to AstraZeneca, but their antibody titers became similar in 3 months. Both vaccines caused systemic AEFIs, and the severity was moderate in the AstraZeneca group compared to Sinovac.

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Home > Archive > v.12(3); Jul 2023

Volume 12(3); Jul 2023

Review Articles

Perspective vaccines for emerging viral diseases in farm animals

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Salalli R, Dange JR, Dhiman S, Sharma T.

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Vaccine hesitancy: acceptance of COVID-19 vaccine in Pakistan

Qazi SH, Masoud S, Usmani MA.

Clin Exp Vaccine Res. 2023 Jul;12(3):209-215. English. Original Article. Open Access

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The issue of vaccine refusal: the study of a risky behavior

Osadchuk M, Tikhonova Y, Krivetskaya M.

Clin Exp Vaccine Res. 2023 Jul;12(3):216-223. English. Original Article. Open Access

Published online July 31, 2023. <https://doi.org/10.7774/cevr.2023.12.3.216>

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Anti-SARS-CoV-2 receptor binding domain antibodies after the second dose of Sinovac and AstraZeneca vaccination

Gondokesumo ME, Purnamayanti A, Hanum PS, Santosa WN, Wardhana AP, Avanti C.

Clin Exp Vaccine Res. 2023 Jul;12(3):224-231. English. Original Article. Open Access

Published online July 31, 2023. <https://doi.org/10.7774/cevr.2023.12.3.224>

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Immunogenic characterization of AlPO₄ adsorbed Td vaccine and liposome-mediated Td vaccine

Shuhsadhe R, Vazhayil J, Ali HS, Orsud H, Mergani AE.

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Published online July 31, 2023. <https://doi.org/10.7774/cevr.2023.12.3.232>

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Factors associated with parental intention to vaccinate their preschool children against COVID-19: a cross-sectional survey in urban area of Jakarta, Indonesia

Santi T, Hegar B, Munasir Z, Prayitno A, Werdhani RA, Bandar IN, Jo J, Uswa R, Widia R, Vandenplas Y.

Clin Exp Vaccine Res. 2023 Jul;12(3):240-248. English. Original Article. Open Access

Published online July 31, 2023. <https://doi.org/10.7774/cevr.2023.12.3.240>

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Antibody response to COVID-19 vaccination in patients on chronic hemodialysis

Choi H, Han S, Kim JS, Park B, Lee MJ, Shin GT, Kim H, Kim K, Park AY, Shin HJ, Park I.

Clin Exp Vaccine Res. 2023 Jul;12(3):249-259. English. Original Article. Open Access

Published online July 31, 2023. <https://doi.org/10.7774/cevr.2023.12.3.249>

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Exploring the essential need for developing COVID-19 vaccines in the Philippines

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Is SARS-CoV-2 vaccination related Guillain-Barré syndrome really different from that due to other causes?

Finsterer J.

Clin Exp Vaccine Res. 2023 Jul;12(3):262-263. English. Correspondence. Open Access

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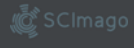
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