Clinical Research

Safety and immunogenicity of the CoV2-Bio in a healthy population aged 18 years and older in Indonesia

Suzy Maria[1], Ciho Olfriani[2], Sukamto Koesnoe[1], Rini Sekartini[2], Soedjatmiko[2], Bernie Endyarni Medise[2], Irene Yuniar[2], Wahyuni Indawati[2], Angga Wirahmadi[2], Rini Mulia Sari[3], Nuri Purwito Adi[4], Windri Retnaningdyah[2]

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Authors' affiliations:
[1]Department of Internal Medicine, Faculty of Medicine, Universitas Indonesia, Cipto Mangunkusumo Hospital, Jakarta, Indonesia, [2]Department of Child Health, Faculty of Medicine, Universitas Indonesia, Cipto Mangunkusumo Hospital, Jakarta, Indonesia, [3]PT. Bio Farma, Bandung, Indonesia, [4]Department of Community Medicine, Faculty of Medicine, Universitas Indonesia, Cipto Mangunkusumo Hospital, Jakarta, Indonesia

Corresponding author:
Suzy Maria
Department of Internal Medicine, Faculty of Medicine, Universitas Indonesia, Cipto Mangunkusumo Hospital, Jalan Pangeran Diponegoro No. 71, Kenari, Senen, Central Jakarta 10430, DK Jakarta, Indonesia
Tel/Fax: +62-21-3141160/ +62-21-3904546
E-mail: suzyduri@gmail.com

ABSTRACT

BACKGROUND According to the WHO Target Product Profiles for COVID-19 Vaccines, vaccine development should be indicated for active immunization in all populations, in conjunction with other control measures to curtail the pandemic. Several RBD-based COVID-19 vaccines are being evaluated and have shown advantages. CoV2-Bio was developed based on the wild-type SARS-CoV-2 RBD amino acid sequence, representing residues of the spike protein of the Wuhan-Hu-1 isolate. This study aimed to evaluate the safety and immunogenicity of CoV2-Bio when compared to CoronaVac.

METHODS This was an observer-blinded, randomized controlled prospective study of safety and immunogenicity of the CoV2-Bio in healthy adult population. A total of 54 healthy participants were randomized to receive either 3 doses of CoV2-Bio or 2 doses of CoronaVac, and 1 dose of placebo, administered 28 days apart. Participants were followed up for safety and immunogenicity. IgG antibody titers (ELISA) and neutralization assay against Wuhan and Delta strains were evaluated at baseline, Days 28, 56, and 84. We assessed seropositive rate, seroconversion, and GMT as parameters.

RESULTS Both vaccines were well tolerated and induced good antibody response. The incidence rate and intensity of local and systemic adverse events did not differ between vaccine and control groups. The vaccine group showed a larger proportion of seroconversion (4-fold increase antibody) (87.5% *versus* 46.2%, $p = 0.001$) and higher GMT (305.9 AU/ml *versus* 102.4 AU/ml, $p<0.001$) when compared to control group.

CONCLUSIONS 3 doses of the CoV2-Bio are safe and immunogenic in healthy adult population. 3 doses of the CoV2-Bio COVID-19 vaccine produce a better immunogenicity profile compared to CoronaVac.

KEYWORDS CoronaVac, COVID-19 vaccine, protein subunit vaccine

Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has triggered a global pandemic, resulting in 6.8 million deaths worldwide. Investments in SARS-CoV-2 vaccine development have led to the safe and affordable COVID-19 vaccines availability, particularly for low- and middle-income countries.[1–3] Producing domestic vaccines and implementing effective administration programs are essential for increasing vaccine coverage and optimizing COVID-19 mitigation costs.[1–3]

Several receptor-binding domain (RBD)-based COVID-19 vaccines have been evaluated in clinical trials and have shown advantages,[4–6] including temperature stability.[7–10] However, their ability to protect against new variants of SARS-CoV-2 is unknown. Additionally, the RBD is a key biomarker and dominant target for the elicitation of

neutralizing antibodies following natural infection or vaccination.[7–10]

Regarding mass production, the use of *Pichia pastoris* as a low-cost expression platform results in high-yield expression of antigens with high purity and a well-defined structure, and can be easily scaled from pilot to industrial-scale manufacturing. The antigen is produced using cyclic guanosine monophosphate and can be placed into vials to generate 20,000 to 200,000 vaccine doses. This is supported by the availability of expertise in fermentation technology using *P. pastoris* for vaccine manufacturing in developing countries, including Indonesia. The production of RBD-based vaccines is cost-effective.

To help overcome the COVID-19 pandemic, Bio Farma Pharmaceutical Company developed a vaccine using a recombinant protein subunit platform.[1,8–11] Bio Farma's currently manufactured protein subunit vaccine, CoV2-Bio, does not require genetic material, making it noninfectious or nonviable. Bio Farma believes in the vaccine's safety and its suitability for mass production.[7]

Several vaccines currently use the same platform as our vaccine candidate, including the ZF2001[12] and the Abdala[13] vaccine. The ZF2001 vaccine, developed by Anhui Zhifei Longcom Biologic Pharmacy Co., Ltd and the Institute of Medical Biology of the Chinese Academy of Medical Sciences, has reached phase 3 clinical trials with promising results.[14] Similarly, the Abdala vaccine, developed by Center for Genetic Engineering and Biotechnology in Cuba, has also shown promising results.[15] These vaccines use aluminum hydroxide as an adjuvant to enhance immunogenicity, as does CoV2-Bio.[13]

Other RBD-based vaccines, such as Corbevax and Covovax, have also demonstrated high safety and immunogenicity.[16,17] Our vaccine candidate, CoV2-Bio, was the first RBD-based COVID-19 vaccine developed in Indonesia at the time the trial was conducted. Bio Farma Pharmaceutical Company also developed another RBD-based vaccine, Indovac, which has already reached phase 3 clinical trials and has shown promising results.[18]

Corbevax, Covovax, and Indovac use similar platforms as our vaccine candidate, CoV2-Bio. The difference is that our vaccine candidate formula contains aluminum hydroxide as an adjuvant, whereas Corbevax, Covovax, and IndoVac also contain cytosine phosphoguanine and aluminum hydroxide. This study aimed to evaluate the safety and immunogenicity of the CoV2-Bio in healthy individuals aged 18 years and older in Indonesia.

METHODS

This observer-blind, comparative, randomized, preliminary study has been registered in ClinicalTrials.gov ID: NCT05067894.

Sample size and population

The initial vaccine testing involved 54 healthy adults aged 18 years and older to focus on clinical tolerance and safety. Participants were divided into vaccine and control groups, with 27 individuals in each group. Participants in the vaccine group received three doses of the vaccine candidate (50 µg of CoV2-Bio), while the control group received two doses of the control vaccine (CoronaVac) and one dose of placebo (normal saline injection).

Procedure

Before participant enrollment, we performed an initial screening of all candidates to determine their eligibility for the study. All candidates underwent the following safety examinations: routine biochemical and hematological tests, urine tests, chest X-ray, electrocardiography, and SARS-CoV-2 antigen detection.

The exclusion criteria included a history of vaccination with any investigational COVID-19 product during or within 6 months before enrollment; a history of COVID-19 within the last 3 months; a positive result on a COVID-19 rapid antigen test; a history of immunodeficiency or uncontrolled chronic disease; women who were pregnant, lactating, or planning a pregnancy during the study period; abnormal hematological or biochemical test results; a history of asthma; or a history of allergies to vaccines or vaccine ingredients.

Each included participant was assigned a number from 001–054 and a randomization code (A/B), which were allocated by the unblinded team. The team randomized and vaccinated the participants using the doses specified in the protocol for each treatment arm. The randomization list was generated automatically using randomization software provided by www.sealedenvelope.com.

The recruitment process was conducted using the age-escalating method, starting with the adult

participants (n = 30). We assessed and reviewed the vaccine safety during the first 7 days after the first vaccination, which showed significant results. Subsequently, the study continued with recruiting elderly participants (n = 24).

The vaccine group received the vaccine candidate (CoV2-Bio), and the control group received the CoronaVac. Three doses of either vaccine were administered on the first visit (V1, Day 0); the second visit (V2, Day 28) within a window period of 4 days after Day 28, and the third visit (V3, Day 56) within a window period of 4 days before and 7 days and after Day 56.

Safety measurements

All participants were observed for 30 min until 28 days following vaccine administration. After each injection, all participants were observed for 30 min to evaluate any immediate adverse event. We had prepared an emergency kit at the vaccination station to anticipate any serious adverse event. Participants were advised to record daily any adverse events, such as local or systemic reactions, in a diary card for 28 days after the last dose. The adverse events to be recorded were listed in the diary card (solicited adverse events), and the participants could also note any medical reactions not listed (unsolicited adverse events).

The safety data for each visit obtained from the diary cards were evaluated and participants were examined by investigators (SM, SK, RS, BEM, IY, WI, and AW) at the next visit (V2, V3, and V4). The solicited local adverse events were pain, redness, induration, and swelling. Pain was graded into mild (pain at the injection site when touched), moderate (pain with movement), and severe (significant pain at rest). Redness, induration, and swelling intensity were measured using a plastic bangle and categorized into mild (<5 cm), moderate (5–10 cm), and severe (>10 cm). Other local events were graded into mild (no interference with activity), moderate (some interference with activity not requiring medical intervention), and severe (limited daily activity requiring medical intervention).

The solicited systemic adverse events were fever, fatigue, and myalgia. Fever was graded into mild (38.0–38.4°C), moderate (38.5–38.9°C), and severe (≥39.0°C). Fatigue, myalgia, and other systemic events were graded into mild (no interference with activity), moderate (some interference with activity not requiring medical intervention), and severe (limited daily activity requiring medical intervention).

Any medical office visit, emergency room visit, or hospitalization for any reason was recorded throughout the trial period. Participants were told to report serious adverse events immediately, and these were documented in the case report form (CRF). All data were recorded in the electronic CRFs and given to the ethics committee. Serious adverse events were reviewed by the Data Safety Monitoring Board (DSMB). Finally, we analyzed the data using SPSS software version 25 (IBM Corp., USA). For safety data analysis, chi-square test and Fisher's exact test were used for comparing the safety variables between groups. A *p*-value of <0.05 was considered a significant difference.

Immunogenicity measurements

Blood samples were taken from all participants at baseline (V0), Day 28 (V2), Day 56 (V3), and Day 84 (V4). These titers were evaluated using a chemiluminescent magnetic microparticle immunoassay for IgG antibody and neutralization assay. We also measured neutralizing antibody (NAb) titer using a modified cytopathogenic effect assay using the wild virus. At the time of this study, the Delta variant of SARS-CoV-2 was the main concern, thus NAb assay was conducted against both the Wuhan and Delta strain. An NAb titer of 1:4 or higher indicated seropositivity.

The seropositive rate and geometric mean titer (GMT) were evaluated at baseline, Days 28, 56, and 84, while the seroconversion rate was determined at baseline, Days 56, and 84. The specific IgG antibodies were measured using the enzyme-linked immunosorbent assay (ELISA) method.

Data analysis was conducted as follows: 1) GMT result was compared after log-transformation, 2) 95% confidence interval, $p<0.05$ was considered a significant difference, 3) seropositivity was defined as titer ≥50 arbitrary unit/ml, 4) seroconversion was defined as either a change from seronegative to seropositive or as 4-fold increase in anti-RBD antibody IgG titer (ELISA) on Days 28, 56, and 84 compared to baseline. The endpoint will be evaluated for specimens with a high titer by retesting them with a higher starting reciprocal dilution. Chi-square and Fisher's exact test were used to compare the proportion of

participants with seropositive and seroconversion between the vaccine and control groups. Mann–Whitney *U* test was used to compare the GMT result between vaccine and control groups.

Ethics approval

The study protocol and all amendments were reviewed and approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia – Cipto Mangunkusumo Hospital (No: KET-845/UN2.F1/ETIK/2021) in compliance with local law (No: 845/UN2.F1/ETIK/PPM.00.02/2021). This trial followed the latest Edinburgh, Scotland revision of the Declaration of Helsinki, The International Council for Harmonisation Good Clinical Practice guidelines, and local regulatory requirements.

Informed consent

All participants had given written informed consent before any study-related procedures were performed, ensuring they were informed of the nature of the trials, the potential risks, and the participants' obligations.

Study intervention

Vaccine candidate

The dose of CoV2-Bio for all participants was 0.5 ml injected intramuscularly to the left deltoid region three times at an interval of 28 days. Every 0.5 ml of vaccine contained 50 µg SARS-CoV-2 recombinant RBD protein subunit, 200 µg aluminum as an adjuvant, 2,742 mg normal saline, and 1,137 mg tris (hydroxymethyl) aminomethane with excipient: Alhydrogel® adjuvant, sodium chloride, and tris (hydroxymethyl) aminomethane. These products are packaged in single-dose prefilled syringes with a minimum recoverable volume of 0.5 ml (0.5 ml/dose).

The CoV2-Bio has SARS-CoV-2 RBD as an antigen. The clone of RBD protein was generated by the Texas Children's Hospital Center for Vaccine Development at Baylor College of Medicine, USA. The protein was developed based on the wild-type SARS-CoV-2 RBD amino acid sequence, representing residues 331–549 of the spike (S) (GenBank: QHD43416.1) protein of the Wuhan-Hu-1 isolate (GenBank: MN908947.3).

This vaccine is a noninfectious recombinant RBD protein of SARS-CoV-2. The recombinant proteins are produced through fermentation in recombinant *P. pastoris*. The fermentation process involves the growth of *P. pastoris* on chemically defined fermentation media that contain glycerol, vitamins, and mineral salts. The recombinant proteins are collected from centrifuged culture supernatant and purified by a series of chemical and physical methods, including chromatography and diafiltration. The purified RBD recombinant proteins are aseptically formulated with buffer, and aluminum hydroxide adjuvant is subsequently added into single-dose prefilled syringes to finalize the product, which is a sterile white liquid suspension.

Control product

The control product is a combination of the COVID-19 Vaccine Bio Farma (CoronaVac) and a placebo. At the time this study was conducted, there were 10 vaccines under emergency use authorization. However, there was no vaccine with the same platform as our vaccine candidate, who has received emergency use authorization. Therefore, we used CoronaVac as a comparator, considering that it was the most extensively used COVID-19 vaccine at that time.

CoronaVac was administered at V1, V2, and followed by placebo at V3. The product was manufactured through inoculation of novel coronavirus (CZ02 strain) into African green monkey kidney (Vero) cells. The virus was incubated, harvested, inactivated, concentrated, purified, and adsorbed by aluminum hydroxide. The result was a milky white suspension, which was stratified by precipitation and easy to shake. The products were packaged in multidose vials (0.5 ml/dose).

Each dose of CoronaVac in the control product contained 600 standard units/0.5 ml (3 g/0.5 ml) of SARS-CoV-2 antigen with excipients, such as aluminum hydroxide, disodium hydrogen phosphate, sodium dihydrogen phosphate, and sodium chloride.[19]

The placebo, which was also produced by Bio Farma Pharmaceutical Company, was a Bacillus Calmette-Guérin vaccine solvent comprising normal saline given by injection. The product was a colorless, odorless, and tasteless liquid packaged in multidose vials (0.5 ml/dose).

RESULTS

Demographics of the study participants

Overall, 54 healthy participants aged 18 years and older were classified into vaccine and control groups,

Table 1. Demographic data

Parameter	Vaccine group (N = 27)	Control group (N = 27)	Total (N = 54)
Age (years), mean (SD)	47.33 (18.381)	51.89 (18.408)	49.61 (18.365)
Sex, n (%)			
Male	12 (44)	13 (48)	25 (46)
Female	15 (56)	14 (52)	29 (54)
History of COVID-19 vaccination prior to study >6 months, n (%)			
Yes	18 (67)	16 (59)	34 (63)
No	9 (33)	11 (41)	20 (37)
History of COVID-19 prior to study >3 months, n (%)			
Yes	5 (19)	1 (4)	6 (11)
No	22 (81)	26 (96)	48 (89)
History of controlled comorbidity, n (%)			
Hypertension	5 (19)	9 (33)	14 (26)
DM	1 (4)	1 (4)	2 (4)
Stroke	0 (0)	1 (4)	1 (2)

COVID-19=coronavirus disease 2019; DM=diabetes mellitus; SD=standard deviation

and of these, 29 (54%) were women. The rest of the demographic characteristics of the participants are shown in Table 1.

Fifty participants (93%) were successfully monitored up to 28 days after the third dose. Four participants terminated the study early: one participant from the vaccine group due to change in domicile, and three from the control group: one lost to follow-up, and the other two was discontinued by the investigator due to adverse event, which are COVID-19 and acute coronary syndrome, respectively.

Safety of CoV2-Bio

We evaluated the number of adverse events and the percentage of participants who developed adverse events. Statistical tests were performed to compare the number of participants who experienced adverse events in the vaccine and control groups.

The overall incidence of adverse events was 63.0% from the first to 28 days after the last dose. The incidence rates of adverse events in the vaccine and control groups were 66.7% and 59.3%, respectively, and the incidence rates did not differ significantly between these groups (p = 0.573). Several adverse events were reported within 7 days after an injection (p = 0.573).

The most frequently solicited adverse events were local pain and myalgia, while the most frequent unsolicited adverse event was influenza. The intensity of most adverse events was mild. Two local reactions, swelling and induration were categorized as severe intensity based on plastic bangle measurements. However, these local reactions were transient and self-limiting.

One moderate unsolicited adverse event, a subcutaneous hematoma, was recorded in the control group and considered unrelated to the vaccination. Moreover, one participant in the control group experienced a serious adverse event; however, after examination and consultation with a specialized doctor and the DSMB, it was deemed unlinked to the vaccination. The data for adverse events after each vaccination in the vaccine and control groups are presented in Figure 1.

Immunogenicity of CoV2-Bio

IgG antibody titer (ELISA)

A comparison of the seropositive rate between the vaccine and control groups revealed no statistically significant difference on Days 28, 56, and 84. However, seroconversion (4-fold increase antibody) and GMT revealed a significant difference in IgG antibody titer at several time points.

On Day 28, compared to the control group, the vaccine group revealed a significantly larger proportion of seroconversion (4-fold increase antibody) (90.0% *versus* 37.5%, p<0.001). On Day 56, when compared to the control group, the vaccine group demonstrated a significantly larger proportion of seroconversion (4-fold increase antibody) (100.0% *versus* 53.3%, p = 0.001) and higher GMT (19,047.6 *versus* 4,326.0, p<0.001). On Day 84, when compared to the control group, the vaccine group indicated a larger proportion of seroconversion (4-fold increase antibody) (100% *versus* 53.33%, p = 0.001) and higher GMT (20,922.6 *versus* 4,138.4, p<0.001). The ELISA result is presented in Figure 2.

NAb against the Wuhan strain

When comparing the seropositive rate between the vaccine and control groups, there was no statistically significant difference on Days 28, 56, and 84. However, seroconversion and GMT had shown a statistical difference in NAb against the Wuhan strain at several time points.

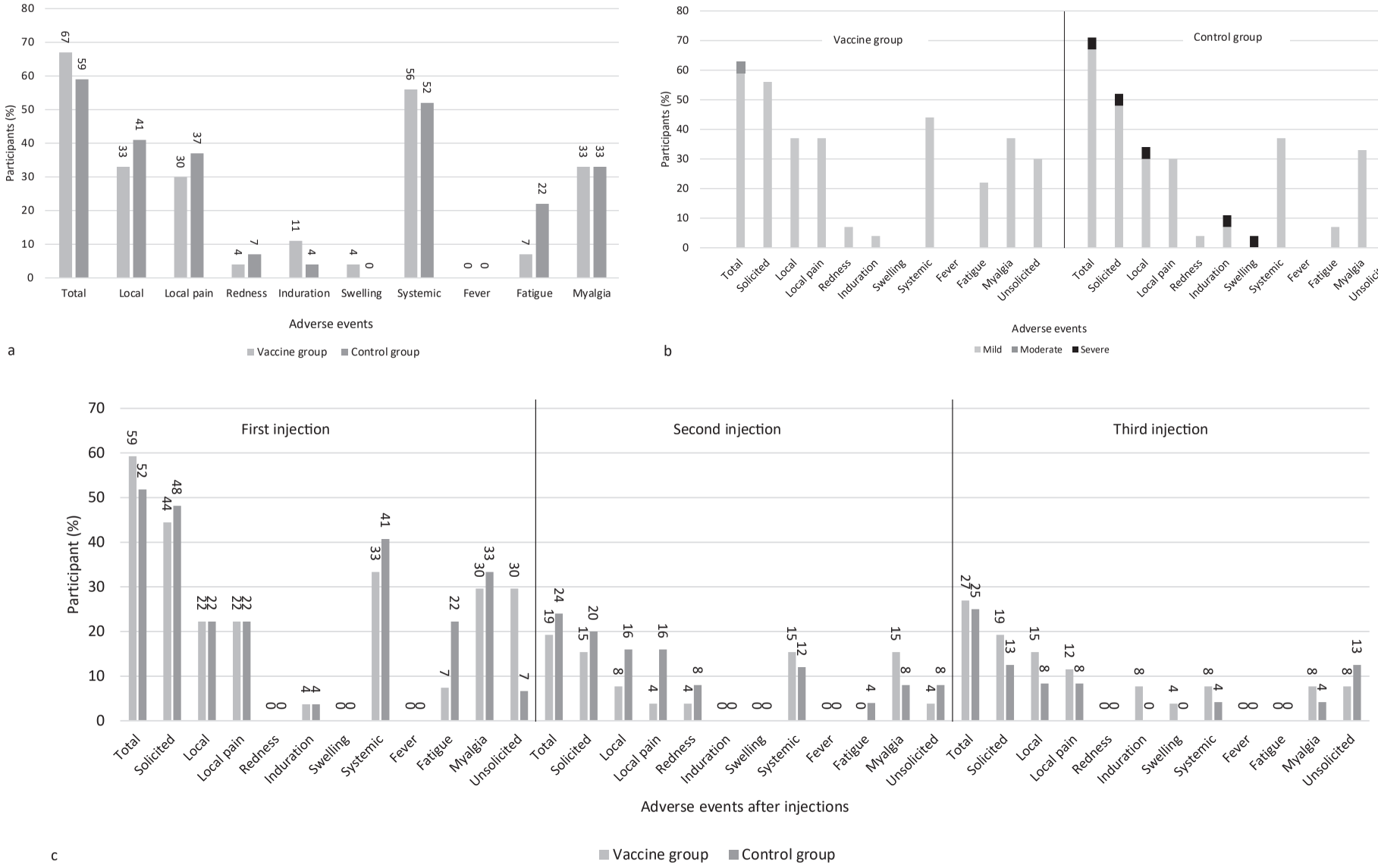

Figure 1. Safety evaluation of CoV2-Bio between groups. (a) Overall incidence of adverse events; (b) intensity of adverse event; (c) adverse event after injections

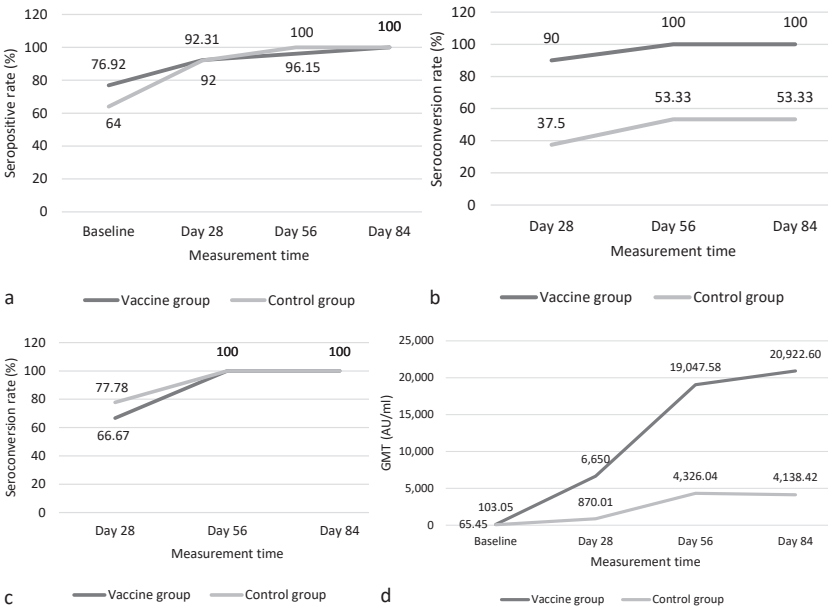

Figure 2. Comparison of anti-RBD antibody IgG titer (ELISA) between groups. (a) Seropositive rate between the control and vaccine groups; (b) seroconversion (4-fold increase antibody) rate between the control and vaccine groups; (c) seroconversion (seronegative to seropositive) rate between the control and vaccine groups; (d) GMT between the the control and vaccine groups. AU=arbitrary unit; ELISA=enzyme-linked immunosorbent assay; GMT=geometric mean titer; RBD=receptor-binding domain

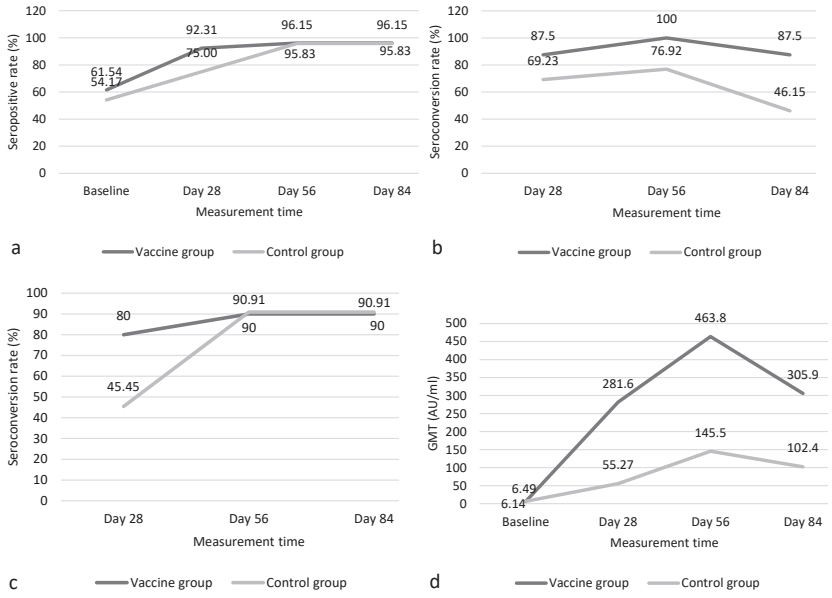

Figure 3. Comparison of neutralization antibody against Wuhan strain between groups. (a) Seropositive rate between the vaccine and control groups; (b) seroconversion (4-fold increase antibody) rate between the vaccine and control groups; (c) seroconversion (seronegative to seropositive) rate between the vaccine and control groups; (d) GMT between the vaccine and control groups. AU=arbitrary unit; GMT=geometric mean titer

On Day 28, the vaccine group exhibited a significantly higher GMT compared to the control group (281.6 *versus* 55.3, p = 0.003). On Day 56, the vaccine group had another significantly higher GMT than the control group (463.8 *versus* 145.5, p = 0.002). On Day 84, the vaccine group showed a larger proportion of seroconversion (87.5% *versus* 46.2%, p = 0.001) and higher GMT (305.9 *versus* 102.4, p = 0.001). The result of NAb against the Wuhan strain is presented in Figure 3.

NAb against Delta strain

There was no statistically significant difference in seropositive rate on Days 28, 56, and 84 between the vaccine and control groups. However, a notable difference between seroconversion and GMT was shown in NAb against Delta strain at several time points.

On Day 28, the vaccine group showed a significantly larger proportion of seroconversion (91.7% *versus* 45.5%, p = 0.027) and higher GMT (163.1 *versus* 31.0, p = 0.004) than the control group. On Day 56, the vaccine group exhibits a significantly larger proportion of seroconversion (100.0% *versus* 54.6%, p = 0.014) and higher GMT (355.6 *versus* 72.4, p = 0.001). On Day 84, the vaccine group showed another larger proportion of seroconversion (100.0% *versus* 63.6%, p = 0.037) and higher GMT (288.1 *versus* 85.7, p<0.001).

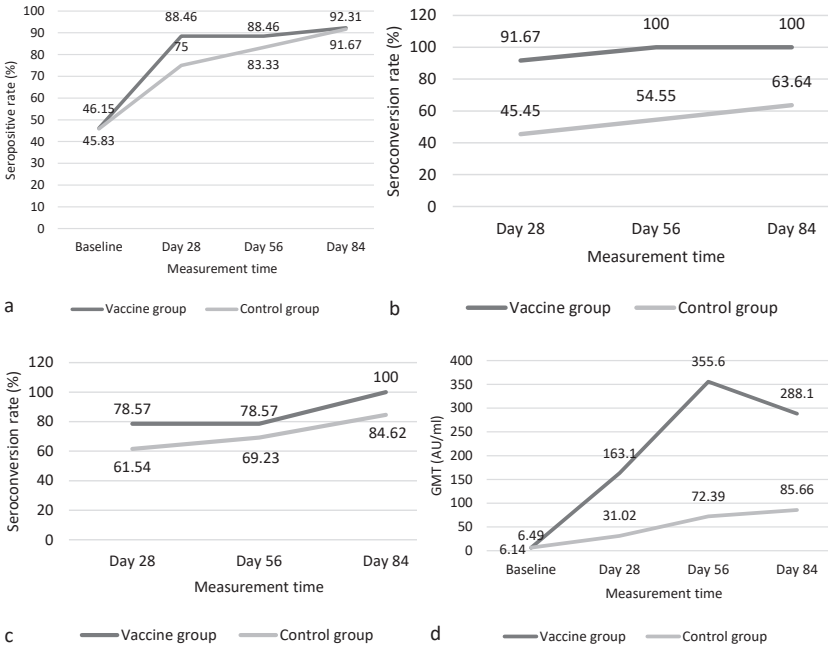

Figure 4. Comparison of neutralization antibody against Delta strain between groups. (a) Seropositive rate between the vaccine and control groups; (b) seroconversion (4-fold increase antibody) rate between the vaccine and control groups; (c) seroconversion (seronegative to seropositive) rate between the vaccine and control groups; (d) GMT between vaccine and control groups. AU=arbitrary unit; GMT=geometric mean titer

Table 2. GMT ratio in V3 and V4 in the vaccine group

Antibody	GMT (AU/ml)		GMT ratio
	Day 56+28 days after the second dose	Day 84+28 days after the second dose	
IgG	19,047.58	20,922.6	1.10
Neutralization antibody			
Wuhan strain	463.80	305.90	0.66
Delta strain	355.60	288.10	0.81

V3=28 days after the second doses; V4=28 days after the third dose

The result of NAb against the Delta strain is presented in Figure 4.

Specifically, comparing immunogenicity data between Days 56 and 84 (two-dose and three-dose vaccination) reveals that the IgG GMT on Day 84 was similar to that on Day 56 (Table 2). The NAb GMT at Day 84 tended to decrease from Day 56. Hence, two doses of recombinant protein subunit vaccine were sufficient to induce an immune response. Further examination in the next phase trial should be conducted to evaluate the immunogenicity data within two doses of vaccine.

DISCUSSION

The trial in this study, which included 54 participants divided into the vaccine and control groups, resulted in a well-performed RBD-based vaccine with minimum deviations and dropouts. A three-dose regimen of CoV2-Bio was well tolerated in healthy adults aged 18 years and older. The incidence rates of adverse events did not differ between the vaccine and control groups. The most frequent local reaction was local pain, and the most common systemic event was myalgia. Most of the adverse events were mild and resolved spontaneously within the first 24–48 hours after onset. These adverse events are anticipated for alum-adjuvanted protein subunit vaccines. Our findings demonstrated that when compared to vaccine control, CoV2-Bio is safe and well-tolerable. This result agrees with previous findings in the safety profile of other similar COVID-19 vaccines.[5–7]

We recorded two severe local reactions—swelling and induration—in the vaccine group; however, these events were transient and self-limiting. The control group experienced one moderate unsolicited adverse event: a subcutaneous hematoma, which was determined to be unrelated to the vaccine after examination. While one participant in the control group had a critical adverse event, based on the examination and expertise of a specialized doctor and the DSMB, we concluded that it was not associated with the vaccination.

Several vaccines resembling our vaccine candidate include the ZF2001 vaccine and the Abdala vaccine, also known as CIGB-66. Both vaccines are based on the recombinant RBD subunit of the spike protein of

the SARS-CoV-2 virus. These vaccines were produced in *P. pastoris* yeast and contain aluminum hydroxide as an adjuvant.[12,13]

In phase three clinical trial, Dai et al[14] included 28,873 participants to analyze the safety and efficacy of the ZF2001 vaccine. They found that the ZF2001 vaccine was safe. The incidence of adverse events and serious adverse events did not differ between the vaccine and control groups, and no vaccine-related deaths were reported. Most adverse reactions were grade 1 or 2.[14]

A phase three Abdala study also demonstrated the potential of delivering safe and protective immune responses of the vaccine against SARS-2 infections. The most frequent adverse event reported mild injection site reactions, which resolved in the first 24–48 hours. No severe adverse events demonstrating a cause-effect relationship to the vaccine were reported.[15]

Regarding immunogenicity, the vaccine group exhibited better immunological performance. The immunogenicity analyses showed a higher seroconversion rates and GMTs of anti-RBD IgG and NAbs against the Wuhan and Delta strains. IgG antibody titer (ELISA) evaluation revealed that the vaccine group had the largest proportion of seroconversion (4-fold increase in antibody titer) after two doses (Day 56) and three doses (Day 84).

A significant difference in GMT was observed when analyzing neutralization against the Wuhan strain. The vaccine group showed a significantly higher GMT than the control group on Days 28, 56, and 84. Notable differences between the vaccine and control groups were also observed in seroconversion and GMT of NAbs against the Delta strain at several time points. The vaccine group exhibited a significantly larger proportion of seroconversion on Days 28, 56, and 84, with the highest seroconversion achieved after two doses. Furthermore, the vaccine group demonstrated a higher GMT than the control group on Days 28, 56, and 84.

Age is a crucial factor influencing the immune response to vaccines, particularly at the extremes of life as in elderly individuals. They often experience a rapid decline in antibody levels, observed in several vaccine studies.[20–22] Given this information, CoV2-Bio has demonstrated a significant difference in immune response among healthy adults and elderly people.

Overall, these findings demonstrated that CoV2-Bio induced a significant immune response in healthy adults and the elderly than the control group. Our findings align with results from a phase one study of the ZF2001 vaccine[12] and a phase one Abdala study[13]. After three doses of the ZF2001 vaccine, the 25 µg and 50 µg vaccine groups demonstrated neutralizing GMTs that exceeded the level of convalescent serum samples obtained from hospitalized patients. The Abdala vaccine induced a significantly higher seroconversion rate in the vaccine group than in the placebo group. This is consistent with Harimurti et al,[23] who compared the anti-RBD IgG levels of Indovac and CoronaVac. The study showed a significant difference in anti-RBD SARS-CoV-2 IgG titers between the vaccine and control groups.

Additionally, we assessed the immunogenicity data between Days 56 and 84 for both two-dose and three-dose vaccinations. The results revealed that two doses of CoV2-Bio induced a better immunogenic response than three doses. The IgG GMT on Day 84 was similar to that on Day 56. Furthermore, the NAb GMT on Day 84 showed a tendency to decrease than Day 56. This finding aligns with other studies of protein subunit vaccines, such as Corbevax and IndoVac, which demonstrated that anti-RBD IgG concentrations plateau after the second dose.[18]

This study has several limitations. The sample size might be minimal to detect potential adverse events. Larger sample sizes may provide significant precision in estimating adverse events. Additionally, we only used a single arm of investigational product, whereas comparing multiple doses of vaccine candidates is typically necessary to determine the most effective dosage.[24] Varying results regarding the effective dose of RBD-based COVID-19 vaccines exist. Yang et al[12] used multiple doses in their study and found that increasing the antigen dose from 25–50 µg did not improve immunogenicity.

Conversely, in the Abdala study, the seroconversion rate in the 50 µg group was significantly higher than in the 25 µg group. Based on the CoV2-Bio preclinical study conducted by Bio Farma Pharmaceutical Company, the 25 µg dose was found to be suboptimal. Therefore, we used only the 50 µg dose of this vaccine candidate in this study. Furthermore, we did not assess T-cell responses in this trial, although they are considered a critical component of immune protection against SARS-CoV-2.[25] In conclusion, three doses of CoV2-Bio are safe and elicit a better immune response against SARS-CoV-2 in a healthy population aged 18 years and

older in Indonesia than two doses of CoronaVac. Our findings demonstrate that CoV2-Bio is a promising candidate and warrants further testing with a larger participant group.

Conflict of Interest

The authors affirm no conflict of interest in this study.

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