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LETTER TO THE EDITOR

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The level of Ig anti-RBD SARS-CoV-2 after two doses of CoronaVac vaccine

The safety and immunogenicity of the two-dose injections of the CoronaVac vaccine (Sinovac Life Sciences) have been reported in Phase 1 and 2 trials.[1,2] After the approval of CoronaVac by the Indonesian government, the coronavirus disease 2019 (COVID-19) vaccination program was launched in January 2021, with the first administration batch targeted towards healthcare workers. However, published data regarding the humoral immune response after CoronaVac vaccination in subjects not included in clinical trials are scarce. Therefore, this report aims to evaluate the effectiveness of two doses of CoronaVac vaccine by investigating the level of total Ig anti-receptor binding domain (RBD) severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

This is a retrospective study carried out using nonprobability sampling. Subjects were included based on the fulfillment of the following criteria: (1) healthy volunteers; (2) healthcare workers receiving CoronaVac vaccine between January and March 2021; (3) has received two doses of CoronaVac (at a 2-week interval [0 and 14 days], with each dose containing inactivated SARS-CoV-2 virus 3 mcg [600 SU] in 0.5 ml); (4) has had a measurement of the Ig anti-RBD quantified by Elecsys Anti-SARS-CoV-2 S (Roche, cut-off value ≥ 0.8 U/ml) after the second dose of CoronaVac vaccine. The Elecsys Anti-SARS-CoV-2 S serology assay is a quantitative electrochemiluminescence immunoassay (ECLIA) utilizing a recombinant protein representing the RBD of the spike antigen and captures predominantly anti-SARS-CoV-2 IgG.[3,4] This study was approved by the Faculty of Medicine and Health Science, Maulana Malik Ibrahim State Islamic University of Malang ethics committee (Ref. No. 023/EC/KEPK-FKIK/2021), and informed consent was obtained from all subjects.

The Ig anti-RBD measurement time was varied, ranging from 7 to 49 days after the second dose of the CoronaVac vaccine. Subjects were hence grouped according to the time of Ig anti-RBD measurement. The Ig anti-RBD level between groups was statistically analyzed either by one-way analysis of variance (ANOVA) or paired/unpaired *t*-test using SPSS version 25 with a significance threshold of $p < 0.05$, and all figures were generated using the Graphpad PRISM.

Initially, a total of 131 subjects, consisting of 59 male (45%) and 72 female (55%) healthcare workers (mean age overall, 35.07 years old [range, 20–67]), were included (Table S1). Among them, 9.9% ($n = 13$) had a history of COVID-19 before vaccination. Among healthcare workers without a history of COVID-19, 96.6% ($n = 114$) had a positive Ig anti-RBD, while only four cases (3.4%, Case number 15, 45, 109, and 112 [27, 51, 42, and 60 years old, respectively]) exhibited a negative Ig anti-RBD after two doses of CoronaVac. One subject (Case number 80 aged 48 years old, Table S1) initially had a negative Ig anti-RBD, but then converted to seropositive at Day 28 (Table S1). Among subjects with a positive Ig anti-RBD, twelve subjects had sufficient Ig anti-RBD levels based on the criteria published by the Food and Drug Administration for the emergency use of convalescent plasma for the COVID-19 treatment (cut-off ≥ 132 U/ml, range from 132.6 to 250 U/ml, subject number 2, 11–13, 17, 38, 61, 66, 102, 105, 100, and 118, see Table S1). Moreover, it is interesting to note that the antibody response was stronger in individuals previously diagnosed with COVID-19 (Table S1).

When the subjects were grouped based on the Ig anti-RBD measurement time at 7, 14, 21, and 28 days after the second vaccination dose, our data revealed that the level of Ig anti-RBD did not differ between groups (71.87 ± 24.69; 52.26 ± 7.35; 48.29 ± 13.17; 54.88 ± 6.44 U/ml, respectively, $p = 0.736$, Figure 1A). No further analysis was performed on the Ig anti-RBD level at 35 ($n = 4$), 42 ($n = 3$), and 49 ($n = 1$) days due to the limited sample size. Further studies with a large sample size are required to confirm this observation.

Twenty-nine subjects had a series of Ig anti-RBD measurements (either on Day 7 and 28 or at Day 14 and 28) after the second dose of the vaccine (Table S1). Fourteen (48%) and 13 (45%) subjects had elevation and suppression of Ig anti-RBD level from baseline, respectively, while two subjects (7%) did not show any changes (Figure 1B). No significant difference was detected between the elevated and suppressed groups in terms of age and sex ($p > 0.05$) (data not shown). Similar to the first analysis (Figure 1A), no significant changes were observed between the level of Ig anti-RBD at Day 7 and 28 or 14 and 28 (89.85 ± 36.26 vs. 73.45 ± 11.15 U/ml, $p = 0.575$; 51.64 ± 7.25 vs. 48.03 ± 7.69 U/ml, $p = 0.490$, respectively, Figure 1C). In addition, no statistically significant gender-based (Figure 1D) or age-based differences were observed neither on Day 14 nor 28 (Figure 1E,F). Interestingly, however, subjects with the age ≥40 years had a lower Ig anti-RBD at Day 14, <30 years old [69.83 ± 16.13 U/ml], 30–39 years old [56.04 ± 8.20 U/ml], ≥40 years old [22.40 ± 8.97 U/ml], p ANOVA = 0.036 (Figure 1E); and tended to have lower Ig anti-RBD at Day 28, 30 years old [59.17 ± 10.87 U/ml], 30–39 years old [69.91 ± 12.57 U/ml], ≥40 years old [33.52 ± 8.29 U/ml], p ANOVA = 0.065; Figure 1F).

In the context of limited data regarding the humoral responses after full-dose administration of CoronaVac vaccine, our preliminary findings show that the majority of subjects had a robust antibody response (with a variable Ig anti-RBD level) from at least 7 days, and was detectable at fairly similar levels 49 days after the second dose

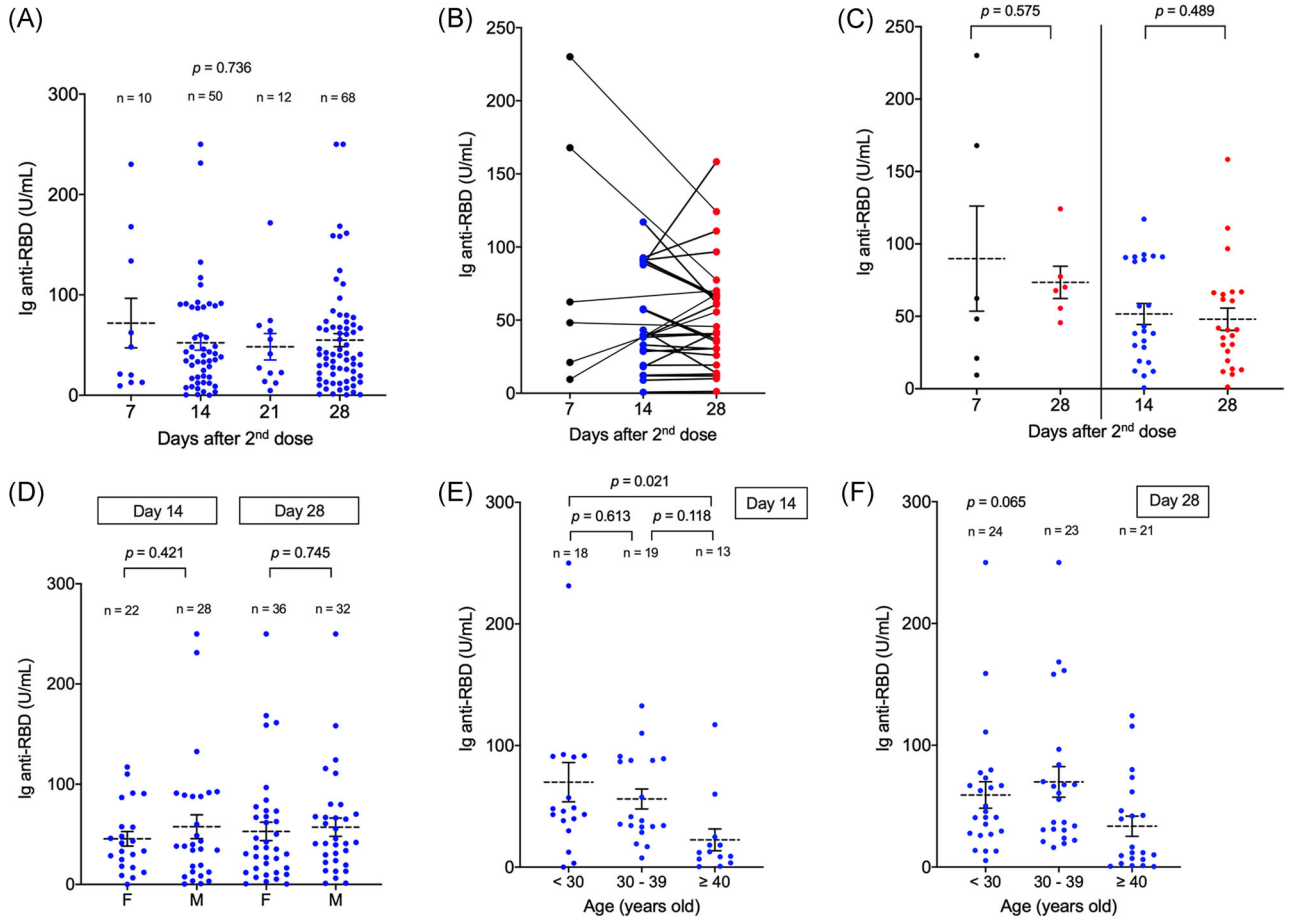

FIGURE 1 Humoral immune response elicited in individuals after receiving the second dose of CoronaVac vaccine. (A) Ig anti-RBD levels at 7, 14, 21, and 28 days postvaccination. Statistical analysis was performed with a one-way analysis of variance (ANOVA) test. (B and C) Ig anti-RBD level from 6 to 23 subjects with the serial measurement at 7 and 28; 14 and 28 days postvaccination, respectively. Statistical analysis was performed with paired *t*-test. (D) Ig anti-RBD level in male or female subjects at 14 and 28 days postvaccination. Statistical analysis was performed with an unpaired *t*-test. (E and F) Ig anti-RBD level stratified by age at 14 and 28 days postvaccination, respectively. Statistical analysis was performed with a one-way ANOVA test. Data presented as mean ± *SEM*

of CoronaVac. We found that among five cases with a negative Ig anti-RBD after the second dose of vaccine, four were older than 40 years old (one case showed seroconversion after 2 weeks). Our data also indicated that subjects older or equal to 40 years old tend to have lower antibody responses towards the CoronaVac. This is in line with the overall notion that aging is associated with a slower and at times suppressed adaptive immune response and that the age-related decline in immunity tends to result in a reduction of a vaccine's prophylactic efficacy.[5,6]

The antibody titers of vaccinees with a pre-existing history of SARS-CoV-2 infection tend to be higher than those without a pre-existing history of SARS-CoV-2 infection and also exceeded the mean of antibody titers evaluated in subjects without a pre-existing history of SARS-CoV-2 infection, similar to previously reported studies utilizing Pfizer BNT162b2 and SPUTNIK V vaccines.[7,8] Nonetheless, the level of Ig anti-RBD stimulated by two doses of CoronaVac in subjects without a history of COVID-19 is much lower than observed in Pfizer BNT162b2 vaccinations as reported by Manisty et al.[9] and Subbarao et al.[10] This is to be expected due to higher efficacy of mRNA vaccines, in general, relative to inactivated virus vaccines.

In addition to the above results, our additional data presented in Table 1 indicated that all patients with breakthrough infection had a similar level of Ig anti-RBD to the current report. However, it is interesting to note that all vaccinated subjects were either asymptomatic or exhibited milder symptoms, except Case number 7, and the symptoms were resolved within 7–14 days, similar to the previous finding.[11] The Ig anti-RBD levels in postinfected individuals were increased (Table 1), but this finding was not observed in Cases 2 and 8, in line with a previous report where patients with symptomatic infection did not respond or had lower antibody levels.[12] In Case number 6, the level of Ig anti-RBD was declined after 3 months and marginally closed to the threshold of positivity, and nearly 7 months later, the subject was reinfected with SARS-CoV-2 (Table 1). One case of the healthcare worker (Case number 104) receiving a third

TABLE 1 Characteristics of breakthrough cases included in this study

Case No.	Sex/ age (y)	Ig anti-RBD level before the vaccine	CoronaVac vaccine		Ig anti-RBD level after the second vaccine	Confirmed SARS-CoV-2	Symptoms	Symptoms resolved during self-isolation	Ig anti-RBD level postinfection
			First dose	Second dose					
1	M/44	ND	Feb 5, 2021	Feb 19, 2021	8.68 U/ml (July 2, 2021)	Aug 4, 2021	Cough, congestion, and headache	Within 7 days	>250 U/ml (Aug 21, 2021)
2	M/33	ND	Feb 5, 2021	Feb 19, 2021	34.2 U/ml (Mar 6, 2021)	July 29, 2021	No symptoms	–	33.74 U/ml (Aug 12, 2021)
3	F/31	ND	Mar 10, 2021	Mar 24, 2021	20.91 U/ml (April 7, 2021)	July 26, 2021	Sore throat, cough, congestion fever, and loss of taste/ smell	Within 10 days	>250 U/ml (Aug 12, 2021)
4	M/26	ND	Mar 10, 2021	Mar 24, 2021	43.26 U/ml (April 7, 2021)	July 28, 2021	Sore throat, cough, fever, and loss of taste/smell	Within 12 days	>250 U/ml (Aug 12, 2021)
5	M/30	ND	Feb 5, 2021	Feb 19, 2021	79.71 U/ml (April 7, 2021)	July 15, 2021	Cough, congestion, and fever	Within 7 days	>250 U/ml (Aug 3, 2021)
6	M/29	ND	Feb 13, 2021	Feb 27, 2021	ND	Aug 20, 2020 (first infection)	No symptoms	–	150 U/ml (Sep 14, 2020) 1.4 U/ml (Jan 4, 2021)
						July 15, 2021 (reinfection)	Sore throat, congestion, and loss of taste/smell	Within 7 days	>250 U/ml (Aug 21, 2021)
7	M/46	ND	Feb 5, 2021	Feb 19, 2021	24.8 U/ml (July 14, 2021)	July 14, 2021	Sore throat, cough, congestion, loss of taste/smell, fever, and pneumonia [admitted to the hospital]	Within 14 days	ND
8	F/26	ND	Feb 3, 2021	Feb 17, 2021	48.74 U/ml (Mar 8, 2021)	April 7, 2021	No symptoms	–	50.16 U/ml (April 20, 2021)
9	M/58	ND	Jan 30, 2021	Feb 13, 2021	6.6 U/ml (July 8, 2021)	Aug 4, 2021	Sore throat, cough, and congestion	Within 7 days	ND

Abbreviations: F, Female; M, Male; ND, not determined; y, year.

dose of CoronaVac (6 months after the second dose) displayed an increase of Ig anti-RBD levels and exceeded the minimal antibody level for the convalescent plasma treatment (2 weeks after the second dose [12.6 U/ml]; 1 month after the third dose [>250 U/ml]). Thus, the potential of periodic booster vaccinations may be required.[13] However, further studies with a larger sample size are needed.

There are some limitations identified in our study. First, our data did not provide Ig anti-RBD level before CoronaVac vaccination. Second, because in this current analysis not all subjects had antibody results at all the time intervals and only subjects with the available data were studied, the results should be interpreted with caution. Further follow-up studies are required to assess the dynamic immune response after the CoronaVac vaccine over a longer time period. In conclusion, although two doses of administration of CoronaVac vaccine elicited the development of humoral immunity against SARS-CoV-2, particularly Ig anti-RBD, the antibody level was mainly lower than the recommended value for the emergency use of convalescent plasma for the COVID-19 treatment or patients who previously had COVID-19 infection.

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