ORIGINAL ARTICLE

CYPRUS
JOURNAL OF MEDICAL SCIENCES

DOI: 10.4274/cjms.2022.2022-10
Cyprus J Med Sci 2022;7(4):438-443

Magnitude of Antibody Response and Risk Factors for Reduced Immunogenicity after Two Doses of an Inactivated Whole-Virion COVID-19 Vaccine (CoronaVac®)

Ersan Berksel[1], Ayşe Ülgen[2]

[1]Department of Nursing and Physiotherapy and Rehabilitation, Cyprus Science University Faculty of Health Sciences, Kyrenia, North Cyprus
[2]Department of Biostatistics, Girne American University Faculty of Medicine, Kyrenia, North Cyprus

Abstract

BACKGROUND/AIMS: CoronaVac® is an inactivated whole-virion Coronavirus disease-2019 vaccine and represents the very first tool in the protective armamentarium in North Cyprus. The aim of this study was to determine the magnitude of anti-severe acute respiratory syndrome-coronavirus 2 spike (S) total immunoglobulin response and the risk factors for reduced immunogenicity post-vaccination.

MATERIALS AND METHODS: Four hundred and twenty-six adults from the general population of North Cyprus were enrolled in this study from the 20th of March 2021 to the 10th of August 2021. The participants involved were actively immunized (with two doses of CoronaVac® at an interval of four weeks).

RESULTS: After the second dose of CoronaVac®, anti-S antibodies were detected in 76% of the participants. Seropositivity was slightly higher among women (77%) than men (75%). Although, intriguingly, seropositivity was found to be highest in both women and men above 65 years, the magnitude of antibody response was moderately negatively correlated with age (r=-0.26), and antibody titers and age were significantly associated ($p<0.001$). Similarly, mean antibody titers for the three age groups studied (18-45, 46-65, >65 years) were significantly different from each other ($p<0.001$), and the magnitude of antibody response decreased as age increased.

CONCLUSION: No statistically significant relationship between self-reported chronic disease status and antibody response to CoronaVac® was found. Quantitation of anti-S antibodies may help facilitate longitudinal monitoring of the humoral response, which can be useful in deciding the dose of CoronaVac® in groups over 65 years of age. This may also help measure the effectiveness of CoronaVac®-based mass vaccination campaigns in yielding collective protection.

Keywords: Antibody titers, CoronaVac, COVID-19, SARS-CoV-2, seropositivity

INTRODUCTION

A previously unheard-of coronavirus crossed the species barrier and emerged as severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2), a novel virus to which humans had no immunity, in Wuhan (Hubei Province, China) in December, 2019. The virus was identified as the causative agent of atypical pneumonia, now called Coronavirus disease-2019 (COVID-19). In spite of containment efforts initiated by public health authorities in China, SARS-CoV-2 rapidly spread across the globe, currently affecting the Americas, Europe, South-East Asia, the Eastern Mediterranean, the Western Pacific, and Africa. COVID-19 was declared a pandemic by the World Health Organization (WHO) on the 11th of March, 2020. As of the 15th of November, 2021, SARS-CoV-2 had

To cite this article: Berksel E, Ülgen A. Magnitude of Antibody Response and Risk Factors for Reduced Immunogenicity after Two Doses of an Inactivated Whole-Virion COVID-19 Vaccine (CoronaVac®). Cyprus J Med Sci 2022;7(4):438-443

ORCID IDs of the authors: E.B. (0000-0003-0528-3911); A.Ü. (0000-0002-0872-667X).

Address for Correspondence: Ayşe Ülgen
E-mail: ayseulgen@gau.edu.tr
ORCID ID: orcid.org/0000-0002-0872-667X

Received: 19.03.2022
Accepted: 04.04.2022

infected over 250 million people worldwide, with over 5 million deaths reported.[1]

Physicians and scientists around the world have been racing to develop vaccines for COVID-19 since the pandemic began. Acquiring immunity through vaccination is important to the community and, in particular, to those people at increased risk of severe COVID-19, including healthcare professionals, the elderly, and those with underlying chronic conditions. To date, several different types of potential COVID-19 vaccines have been developed, such as inactivated or weakened virus vaccines, viral vector vaccines, RNA and DNA vaccines, and protein-based vaccines. Some of them (e.g. DNA and protein-based vaccines) still await to be listed for WHO emergency use listing. As of November 10th, 2021, over 7 billion vaccine doses had been administered worldwide.[1]

In the fight against the COVID-19 pandemic, Turkey's Pharmaceuticals and Medical Devices Agency gave emergency use approval for the use of an inactivated whole-virion COVID-19 vaccine, called CoronaVac® (produced by the Beijing-based pharmaceutical company Sinovac), on the 13th of January, 2020. The first phase of vaccination started on the 14th of January, 2020 with healthcare workers. The same vaccine was included shortly afterward in the national mass vaccination program of the general population of North Cyprus, which is the subject of this report. The WHO validated CoronaVac® for emergency use on the 1st of June, 2021, giving other countries the assurance that it meets international standards for safety, efficacy, and manufacturing.[2]

The efficacy of CoronaVac® against symptomatic SARS-CoV-2 infection and severe COVID-19 (in which hospitalization is required) and its safety among volunteers aged 18-59 was demonstrated in a double-blind, randomized, placebo-controlled phase 3 clinical trial in Turkey.[3] Outside the scarce number of trials, there are limited data on post-vaccine antibody responses in other groups such as older (>65 years) adults. Also, parameters which predict the risk of low or no antibody titers to inactivated SARS-CoV-2 are yet to be defined explicitly. In the present study, we aimed to determine the magnitude of anti-spike total immunoglobulin response and risk factors for reduced immunogenicity post-vaccination in 426 adults from the general population of North Cyprus.

MATERIALS AND METHODS

Type of research: This study was a retrospective one which involved participants who were actively immunized with two doses of CoronaVac® at an interval of four weeks.

Study Design and Population

The study period comprised of nearly 21 weeks from the 20th of March 2021 to the 10th of August 2021, with test carried out at the Erduran Lab in Kyrenia. During this time, the predominant circulating SARS-CoV-2 lineages in North Cyprus were wild-type and the alpha variant of concern (B.1.1.7). The participants involved were actively immunized (with two doses of CoronaVac® at an interval of four weeks), they were both male and female adults (≥18 years of age) who were interested in confirming their own vaccination success based on the detection of anti-spike antibodies. Those participants who had previously had COVID-19 were excluded from this study. Chronic conditions present in the participants were recorded based on their self-reported information.

Ethical considerations: This study was conducted with Cyprus Science University, Ethics Committee's approval (approval number: 2021.12.002). All participants gave their oral consent to participate in this study, which was conducted in accordance with the Declaration of Helsinki.

Vaccine

CoronaVac® is manufactured by Sinovac Life Sciences. Its production cycle involves inoculating SARS-CoV-2 (CZ02 strain) onto African green monkey kidney cells (Vero cells), cultivation, harvest, inactivation, concentration, purification, and aluminum hydroxide adsorption. Each vial contains a single dose of 0.5 mL which is composed of inactivated SARS-CoV-2 (CZ02 strain) at 600 SU (active ingredient), aluminum hydroxide at 0.225 mg (adjuvant), and excipients (phosphate at 0.0025 mmol, sodium chloride at 4.5 mg, and water for injection). The vaccination of the participants was performed in authorized hospitals with the recommended dosing interval of four weeks between the first and second doses, which were administered into the deltoid muscle.

Sample Collection

Blood samples were collected from the participants within 14 to 28 days after the administration of the second dose of CoronaVac®. Participants underwent blood sampling with standard venipuncture at a single center. Serum separator tubes with separating gel and clot activator were used for the blood collection.

Analysis of Samples

The Elecsys® anti-SARS-CoV-2 S immunoassay (Roche) running on a cobas® e801 modular analyzer (Roche) was used for the analysis of the samples collected. Elecsys® anti-SARS-CoV-2 S is a relatively fast (testing time: 18 minutes) one-step double-antigen sandwich assay for the *in vitro* quantitative determination of total antibodies (including IgG) directed against the SARS-CoV-2 spike (S) protein receptor-binding domain in human serum and plasma. The linear quantification range of the assay is between 0.4 U/mL and 250 U/mL, and the manufacturer's stated threshold cut-off for reactivity or positivity is 0.8 U/mL. The clinical sensitivity of the assay is reported to be 98.8%, and its clinical specificity is nearly 100%. (sensitivity estimations are based on a sampling date of ≥14 days after diagnosis with real-time quantitative PCR).

Statistical Analysis

All statistical analyses were carried out by R packages (available at https://www.r-project.org) and functions, ANOVA (analysis of variance), Student's t-test (t-test), cor.test (correlation test), cor (correlation coefficient), wilcox.test (Wilcoxon test), glm (..., family='binomial') (logistic regression). In group comparisons, One-Way analysis of variance (ANOVA) or independent samples t-tests were used for continuous variables and χ^2-test was used for qualitative variables. In all evaluations, $p<0.05$ was considered statistically significant.

RESULTS

Table 1 shows the descriptive statistics of the patients in terms of frequency and percentage values for the different age groups. A total of 426 patients were enrolled at the beginning of this study. The mean age (± standard deviation) of the participants was 57.7 (±16.7) years, and their median age was 61 years old. The minimum age was 18 years, and the maximum age was 91 years old. The cohort had a slightly greater representation from male individuals, with 55% male and 45% female. The age distribution was as follows: 18-45 years old, 113 (27%); 46-65 years old, 159 (37%); 65 years old and above, 154 (36%) (Table 1).

Table 1. The demographic characteristics of the patients according to their age groups			
Patients (%)			
Age (years)	**Female (%)**	**Male (%)**	**Total (%)**
Age (mean ± SD)	55.9 (±16.6)	59.1 (±16.6)	57.7 (±16.7)
18-45 years	57 (30)	56 (24)	113 (27)
46-65 years	73 (38)	86 (36)	159 (37)
>65 years	60 (32)	94 (40)	154 (36)
Total	190 (45)	236 (55)	426 (100)
SD: Standard deviation			

Table 2 shows the quantitative assessment of anti-SARS-CoV-2 S total immunoglobulin seropositivity in the patients 14-28 days after the second dose of the CoronaVac® vaccination. Between 14-28 days, anti-S antibody levels were detectable in 324 out of 426 patients. Seropositivity was slightly higher among females (147/190 or 77.4%) than among males (177/236 or 75.0%), though this difference was not statistically significant. In addition, seropositivity was found to be highest in both women and men above 65 years old (81.7% and 81.9%, respectively). Among patients between 46 and 65 years, anti-S antibodies in females and males were 74% and 75.6%, respectively, and among those between the ages 18-45, it was 77.2% in females and 62.5% in males. There was no statistically significant difference between the age groups of both genders in terms of anti-S antibody seropositivity.

Linear correlation between age and anti-S immunoglobulin levels was -0.26 ($p<0.001$) and 95% confidence interval (-0.36, -0.15). Therefore, there seems to be a low-to-moderate negative correlation between age and antibody levels. Figure 1 shows the linear regression plot of anti-S total immunoglobulin and age. As can be seen from this plot, the association between the two variables is highly significant ($p<0.001$).

Table 3 shows logistic regression results with age for different threshold values for the anti-S total immunoglobulin in binary categories, namely (Ig <251, Ig >251), (Ig <201, Ig >201), (Ig <151, Ig >151), (Ig <101, Ig >101) and (Ig <51, Ig >51). Therefore, the logistic regression results for Ig <201, with Ig >201 as the dependent variable, and age as independent variable are significantly associated ($p=2.537614e-05$). Therefore, we can say that the mean age of the patients in the groups with titers <201 versus titers >201 is the most statistically significant ($p<0.001$).

Pearson's χ^2-test for the three immunoglobulin levels <150, 150-250, >250 for the three age groups (18-45, 46-65, >65 years) was statistically significant ($\chi^2=46.4$ with 4 df; $p<0.001$). Therefore, χ^2-test of independence shows that the three titer groups are independent from the three age groups (Table 4). One-Way analysis of variance (ANOVA) results comparing the three age groups (18-45, 46-65, >65 years) for

Table 2. Quantitative assessment of anti-SARS-CoV-2 S total immunoglobulin seropositivity in patients 14-28 days after their second dose of vaccination with CoronaVac®						
Characteristics of patients (n=324)			**Anti-S antibodies**			
Age by group	**Gender**	**COVID-19 history**	**Antibody state (%)**	**Median**	**Minimum**	**Maximum**
18-45 years, (n=113)	Male	Positive	33	207.65	35	>250
		Negative	2	0	0	0
		Male total	**35 (62.5)**	207.65	35	>250
	Female	Positive	43	250	28	>250
		Negative	1	0	0	0
		Female total	**44 (77.2)**	250	28	>250
		Total	79 (69.9)			
46-65 years, (n=159)	Male	Positive	62	93	15	>250
		Negative	3	0	0	0
		Male total	**65 (75.6)**	92	1	>250
	Female	Positive	52	174	21	>250
		Negative	2	0	0	0
		Female total	**54 (74.0)**	174	21	>250
		Total	119 (74.8)			
>65 years, (n=154)	Male	Positive	72	142.10	35	>250
		Negative	5	0.4	0	0
		Male total	**77 (81.9)**	137.65	0	>250
	Female	Positive	48	183.4	16	>250
		Negative	1	0	0	0
		Female total	**49 (81.7)**	183.4	16	>250
		Total	126 (81.8)			

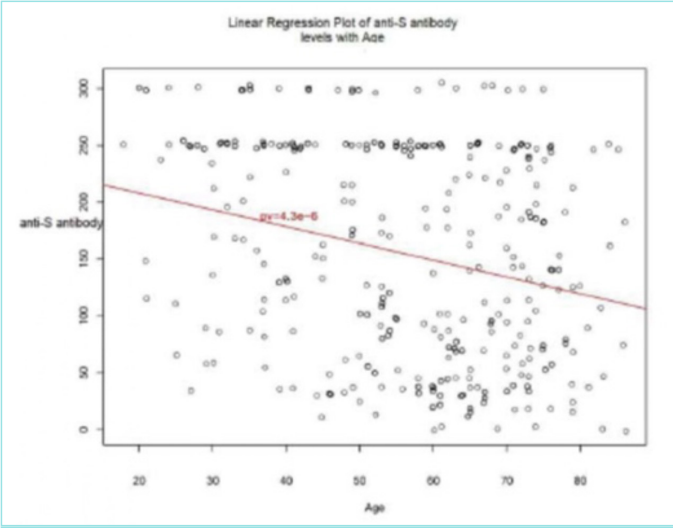

Figure 1. Linear regression plot of anti-S total immunoglobulin and age

anti-S total immunoglobulin levels gives a statistically significant result ($p<0.001$). Therefore, differences in mean immunoglobulin levels for the three age groups are statistically significant. Overall, our findings clearly indicate that as the age increases, immunoglobulin levels decrease.

Table 5 shows the percentage of patients with and without comorbidities. Table 6 shows the additional diseases which the patients had; 6.7% had diabetes mellitus, 15.4% had both diabetes mellitus and hypertension, 41.2% had hypertension, 0.5% had chronic obstructive pulmonary disease, 0.5% had coronary heart disease, 0.5% had rheumatoid arthritis, and 35.2% were not specified. There was no statistically significant relationship between their self-reported chronic disease status and their antibody response to CoronaVac®.

DISCUSSION

The available evidence in the relevant scientific literature suggests that SARS-CoV-2 induces a typical antibody-mediated (humoral) immune response pattern, where IgM is the first antibody to appear, followed closely by IgA (which peaks at two to three weeks post-symptom onset and declines over the following weeks) and finally by IgG which remains elevated for several months post-symptom onset.[4] Therefore, there is a logical need for serological assays which can assess the humoral immunity conferred by infection with SARS-CoV-2 or by vaccination with the currently available COVID-19 vaccines. Neutralization assays, which give information as to whether the detected antibodies can neutralize SARS-CoV-2 and offer potential protection upon subsequent exposure, involve the live authentic virus produced in cell cultures and thus necessitate all procedures to be conducted in a Biosafety Level 3 facility.[5] On the other hand, traditional antibody assays, which measure the reactivity of antibodies in human serum or plasma with virus-specified antigens, can be performed in a standard diagnostic laboratory in a timely and high-throughput manner.

Table 3. Logistic regression results with IgG as dependent and age as independent factors

Threshold IgG	p-value
51	0.004
101	0.0004
151	0.0007
201	**2.537614e-05**
251	0.004

IgG: Immunoglobulin G

From a public health perspective, anti-SARS-CoV-2 antibody assays are indispensable tools for assessing the fraction of people affected by COVID-19 as well as for identifying those who are still at risk of an infection with SARS-CoV-2. In addition, vaccinated people who are interested in confirming their own vaccination protection status also bring increasingly insistent demands for anti-SARS-CoV-2 antibody assays. The SARS-CoV-2 spike (S) protein is highly conserved among all human coronaviruses and participates directly in receptor (angiotensin-converting enzyme 2) recognition, viral attachment, and the entry into host cells. Due to its crucial functions and role in the life cycle of SARS-CoV-2, S protein represents one of the most important targets for both unconventional (modern) vaccine development and therapeutic intervention.[6] Here, we used a commercially available anti-SARS-CoV-2 S total antibody assay to evaluate the immunogenicity of the conventional vaccine CoronaVac®. We note that the threshold cut-off for seropositivity provided by the manufacturer should be considered of diagnostic value only, since it may not always be indicative of absolute protection against SARS-CoV-2 infection.

We showed that CoronaVac® induced robust humoral response only in 76% of patients (324 out of 426), with the resulting antibody titers decreasing with older age in males and females. This could be linked to immunosenescence which leads to an impaired adaptive immune response to vaccinations. Not only the human antibody repertoire, but also the human T-cell receptor repertoire is known to diminish among the elderly, along with a decrease in the relative numbers of naive T-cells and an increase in the relative numbers of memory T-cells in the same age group.[7] Also, we observed lower CoronaVac® immunogenicity in males than in females, albeit falling short of the accepted statistical significance threshold. Indeed, it has been established that, regardless of age, females develop greater antibody responses to vaccinations compared to males, possibly owing to their relatively high basal immunoglobulin levels as well as B-cell numbers.[8]

Table 4. Pearson's χ^2-test results

		Immunoglobulin levels			
		<150	**150-250**	**>250**	
Age groups	18-45 years	42	16	56	114
	46-65 years	99	22	38	159
	>65 years	107	27	20	154
Total		248	65	114	427

Table 5. Additional comorbidities
No additional chronic disease (n=232) (54%)
Additional chronic disease (n=194) (46%)

Table 6. Clinical characteristics of comorbidities						
Additional chronic disease	**DM**	**DM and HT**	**HT**	**COPD**	**CHD**	**RA**
	(n=13)	(n=30)	(n=80)	(n=1)	(n=1)	(n=1)
	6.7%	15.4%	41.2%	0.5%	0.5%	0.5%
DM: diabetes mellitus, HT: hypertension; COPD: chronic obstructive pulmonary disease, CHD: coronary heart disease, RA: rheumatoid arthritis						

As with many other infectious diseases which can be prevented by vaccination, the globally spread SARS-CoV-2 infection may also be controlled by vaccinating a sufficient proportion of the world's population with available and accessible COVID-19 vaccines to achieve herd, or community, immunity. CoronaVac® is an inactive whole-virion COVID-19 vaccine. Inactivated vaccines differ from live attenuated ones in that they are unable to revert to a more virulent phenotype or interfere with each other when combined. CoronaVac® can be kept refrigerated, which facilitates its successful deployment in developing countries. Furthermore, it is available in single-dose vials, which promotes ease of administration and minimizes wastage. Although our findings reveal the presence of non-responders at a ratio of 24%, double-blind, randomized, placebo-controlled phase 2 clinical trials performed by Sinovac Life Sciences demonstrate that receiving a third (booster) dose of CoronaVac® at six months post-vaccination leads to a more than 20-fold increase in quantitative neutralizing antibody response in healthy adults aged 18-59[9] and a more than 30-fold increase in quantitative neutralizing antibody response in older adults.[10]

Study Limitations

One limitation of this study was that it overlooked the presence of truly neutralizing antibodies. The lack of assessment of T-cell responses against CoronaVac® was another limitation of the present study. It could also be instructive to test each subject by means of real-time quantitative PCR prior to the antibody assay since nasopharyngeal-swab PCR positivity, which indicates active SARS-CoV-2 infection, begins to overlap with IgM and IgG seroconversion from the second week of symptom onset.[11]

CONCLUSION

A commercially available anti-SARS-CoV-2 S total antibody assay was used to evaluate the immunogenicity of the conventional vaccine CoronaVac®. It is noted that the threshold cut-off for seropositivity provided by the manufacturer should be considered of diagnostic value only, since it may not always be indicative of absolute protection against SARS-CoV-2 infection. CoronaVac® induced robust humoral response was seen in only 76% of the patients (324 out of 426), with the resulting antibody titers decreasing with older age in males and females, which may be linked to immunosenescence which leads to an impaired adaptive immune response to vaccinations. Lower CoronaVac® immunogenicity in males than in females was also observed, albeit falling short of the accepted statistical significance threshold.

MAIN POINTS

- We used a commercially available anti-SARS-CoV-2 S total antibody assay to evaluate the immunogenicity of the conventional vaccine CoronaVac®. We note that the threshold cut-off for seropositivity provided by the manufacturer should be considered of diagnostic value only, since it may not always be indicative of absolute protection against SARS-CoV-2 infection.
- We showed that CoronaVac® induced robust humoral response in only 76% of the patients (324 out of 426), with the resulting antibody titers decreasing with older age in males and females which could be linked to immunosenescence which leads to an impaired adaptive immune response to vaccinations.
- We observed lower CoronaVac® immunogenicity in males than in females, albeit falling short of the accepted statistical significance threshold.

Acknowledgements: We would like to thank Assoc. Prof. Dr. Kerem Terali for his valuable contributions to this article.

ETHICS

Ethics Committee Approval: This study was conducted with Cyprus Science University, Ethics Committee's approval (approval number: 2021.12.002).

Informed Consent: All participants gave their oral consent to participate in this study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.B., Concept: E.B., Design: E.B., A.Ü., Data Collection and/or Processing: E.B., A.Ü., Analysis and/or Interpretation: A.Ü., Literature Search: E.B., A.Ü., Writing: E.B., A.Ü.

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The author declared that this study had received no financial support.

REFERENCES

1. World Health Organization Coronavirus (COVID-19) Dashboard with Vaccination Data. WHO; (2021a). Available at: https://covid19.who.int/
2. World Health Organization Validates Sinovac COVID-19 Vaccine for Emergency Use and Issues Interim Policy Recommendations [Press Release]. WHO; (2021b). Available at: https://www.who.int/news/item/01-06-2021-who-validates-sinovac-covid-19-vaccine-for-emergency-use-and-issues-interim-policy-recommendations
3. Tanriover MD, Doğanay HL, Akova M, Güner HR, Azap A, Akhan S, et al. CoronaVac Study Group. Efficacy and safety of an inactivated whole-

virion SARS-CoV-2 vaccine (CoronaVac): interim results of a double-blind, randomised, placebo-controlled, phase 3 trial in Turkey. Lancet. 2021; 398 (10296): 213-22.

4. Guo L, Ren L, Yang S, Xiao M, Chang D, Yang F, et al. Profiling early humoral response to diagnose novel Coronavirus disease (COVID-19). Clin Infect Dis. 2020; 71(15): 778-85.
5. Center for Disease Control Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19). CDC; (2020). Available at: https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html
6. Huang Y, Yang C, Xu XF, Xu W, Liu SW. Structural and functional properties of SARS-CoV-2 spike protein: potential antivirus drug development for COVID-19. Acta Pharmacol Sin. 2020; 41(9): 1141-9.
7. Cunha LL, Perazzio SF, Azzi J, Cravedi P, Riella LV. Remodeling of the Immune Response With Aging: Immunosenescence and Its Potential Impact on COVID-19 Immune Response Front Immunol. 2020; 11: 1748.
8. Flanagan KL, Fink AL, Plebanski M, Klein SL. Sex and Gender Differences in the Outcomes of Vaccination over the Life Course. Annu Rev Cell Dev Biol. 2017; 33: 577-99.
9. Pan H, Wu Q, Zeng G, Yang J, Jiang D, Deng X, et al. Immunogenicity and safety of a third dose, and immune persistence of CoronaVac vaccine in healthy adults aged 18-59 years: interim results from a double-blind, randomized, placebo-controlled phase 2 clinical trial. MedRxiv. 2021; 1-25. https://doi.org/10.1101/2021.07.23.21261026
10. Li M, Yang J, Wang L, Wu Q, Wu Z, Zheng W et al. A booster dose is immunogenic and will be needed for older adults who have completed two doses vaccination with CoronaVac: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. MedRxiv. 2021; 1-30. https://doi.org/10.1101/2021.08.03.21261544
11. Sethuraman N, Jeremiah SS, Ryo A. Interpreting Diagnostic Tests for SARS-CoV-2. JAMA. 2020; 323(22): 2249-51.