Original Article

SARS-Cov-2 Antibody Prevalence Among Blood Donors at a Southern Nigerian Teaching Hospital in the Post-Pandemic Context

Iriagbonse Iyabo Osaigbovo[*1, 2], Ehijie Xavier Iyamah[3], Ejime Nicholas Ebeigbe[2], Dorathy Okpokam[3], Elfreda Irabor[3], Maryam Isemobita Eranga-Muhammadkabir[3], Esohe Olivia Ogboghodo[4]

1.Department of Medical Microbiology, School of Basic Clinical Sciences, College of Medical Sciences, University of Benin, PMB 1154, Benin City, Edo State. 2. Department of Medical Microbiology, University of Benin Teaching Hospital, PMB 1111, Benin City, Edo State. 3. Medical Laboratory Services, Haematology and Blood Transfusion Unit, University of Benin Teaching Hospital, PMB 1111, Benin City, Edo State. 5. Department of Public Health and Community Medicine, University of Benin Teaching Hospital, PMB 1111, Benin City, Edo State.

Abstract

Background: Coronavirus disease 2019 (COVID-19) no longer constitutes a public health emergency of international concern but remains a global health threat. However, diminishing testing rates in health facilities constitutes a barrier to effective disease surveillance in Nigeria. **Objective:** To determine the seroprevalence of SARS-CoV-2 antibodies among healthy Nigerian blood donors in the post-pandemic era. **Methods:** A cross-sectional study of consenting voluntary blood donors was conducted in the University of Benin Teaching Hospital, Nigeria between January and April, 2024. A rapid lateral flow device, the Standard Q IgG-IgM COVID-19 rapid test (SD Biosensor, Republic of South Korea) was used to test their blood samples for SARS-CoV-2 IgM and IgG antibodies. Data analysis employed descriptive and inferential statistics with statistical significance set at $p \leq 0.05$. **Results:** Of 274 blood donors tested, the majority, 227 (82.8%) were males, 123 (44.9%) were in the 21-30-year age bracket and blood group O, 213 (77.7%) was predominant. Seventy-five (27.4%) tested positive for IgM, 90 (32.8%) had IgG SARS-CoV-2 antibodies while 34 (12.4%) had both IgM and IgG. Of 75 donors with IgM antibodies, 17 (44.0%) belonged to blood group A. The association between ABO blood group and IgM seropositivity was significant ($p= 0.036$). **Conclusion:** The study demonstrated a notable seroprevalence of SARS-CoV-2 antibodies among Nigerian blood donors indicating ongoing viral circulation and highlighting the association between ABO blood group and recent infections. The findings underscore the importance of serosurveillance in monitoring community-level transmission in the post-pandemic era.

Key words: Seroprevalence; COVID-19; Surveillance; Blood donors; Nigeria

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), a novel coronavirus which originated in Wuhan China in late 2019.[1] The viral outbreak rapidly escalated to a global crisis, earning it the status of a Public Health Emergency of International Concern (PHEIC) by the World Health Organization (WHO) on January 21, 2020.[2] By March 11, 2020, it was declared a pandemic.[3]

The pandemic's scale necessitated widespread diagnostic testing to identify active infections, with molecular detection of SARS-CoV-2 viral genetic

*Correspondence

Dr Iriagbonse Iyabo Osaigbovo
Department of Medical Microbiology,
School of Basic Clinical Sciences,
College of Medical Sciences, University of Benin, PMB 1154,
Benin City, Edo State.
Email: iyabo.osaigbovo@uniben.edu
Phone number: +2348035778626

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

How to cite this article: Osaigbovo *et al:* SARS-Cov-2 Antibody Prevalence Among Blood Donors at a Southern Nigerian Teaching Hospital in the Post-Pandemic Context. Ann Trop Pathol., 2024; 15 (2): 37-41

material as the gold standard.[4] As the pandemic evolved, antigen testing was incorporated to supplement diagnostic capacity.[4] Despite substantial investments in diagnostic infrastructure, actual infection rates were likely underestimated due to high proportions of asymptomatic or mild cases, testing hesitancy, and limitations in testing resources, particularly in low- and middle-income countries.[5]

As a complementary surveillance strategy, seroprevalence studies were undertaken to estimate cumulative exposure and infection-induced immunity. These studies offered insights into population-level virus exposure especially during the early phase of the pandemic prior to the the introduction of vaccines.[6] By detecting antibodies to SARS-CoV-2, seroprevalence surveys provided more comprehensive infection estimates than case-based reporting alone. The conduct of serological surveys via blood donation services presented a feasible approach to monitoring SARS-CoV-2 exposure without the logistical and financial burdens of community-based surveys and was implemented in several countries.[7-10]

As of May 5, 2023, after recording over 700 million confirmed cases and close to 7 million deaths, the WHO announced that COVID-19 no longer constituted a public health emergency but iterated, however, its continued risk to global health.[11] Consequently, surveillance for the disease remains desirable. COVID-19 is included, along with others, as a priority infectious disease requiring routine testing and reporting in Nigeria's integrated disease surveillance and response strategy.[12] Yet persons with asymptomatic or mild disease who contribute significantly to the burden of COVID-19 are unlikely to present for testing.[5] More importantly, testing operations within health facilities across the country have dropped significantly. Serosurveillance provides a practical approach for circumventing the barrier to surveillance posed by this reduction in routine testing activity. The aim of this study is to determine SARS-CoV-2 seroprevalence amongst healthy blood donors during the post-emergency phase of the COVID-19 pandemic.

METHODS

Study Design and Setting

From January to April, 2024, we conducted a cross-sectional study among healthy, voluntary blood donors in the blood bank of the University of Benin Teaching Hospital, Benin City, Edo state. Edo state recorded its first case of COVID-19 in March, 2020. As of February, 2023, the last publicly available update from the Nigeria Centre for Disease Control showed that the state had a total of 7,928 confirmed cases, with 322 deaths and ranked seventh amongst states with the highest COVID-19 burden in the country.[13] During the pandemic, the most severe cases of COVID-19 in Edo state were managed at the University of Benin Teaching Hospital.[14] The hospital's molecular virology laboratory was activated for COVID-19 testing on May 10, 2020 quickly becoming the diagnostic and public health surveillance hub of the state.[15] By the end of the PHEIC on May 5, 2023, a total of 26,694 samples were processed, of which 3535 were positive.[15]

The UBTH blood bank collects blood from voluntary non-remunerated donors, aged 18–60 years old and in general good health, after excluding transfusion transmissible infections including HIV, hepatitis B and hepatitis C. On the average, 300 persons donate blood voluntarily every month.

Ethical Considerations

Ethical clearance for the study was sought and obtained from the Health Research and Ethics committee of the UBTH (Protocol number: ADM/E22/A/VOL. VII/48311703). The blood bank director also granted permission to conduct the study.

Prior to participation, all blood donors were briefed on the study objectives, and only those who provided written informed consent were included.

Sample Size Calculation

Using the formula for sample size determination in prevalence studies and based on the prevalence of 78.9% reported by Kolawole et al,[16] we calculated a minimum sample size of 255.

Data Collection

Demographic and blood group data of consenting donors were collected from the existing blood bank database, with each participant anonymized using a unique patient identifier. Leftover samples from routine blood screening were used for SARS-CoV-2 antibody testing.

SARS-CoV-2 Antibody Testing

Laboratory staff responsible for screening donors for transfusion transmitted infections were trained to test for SARS-CoV-2 antibodies using the Standard Q IgG-IgM COVID-19 rapid test device (SD Biosensor, Republic of South Korea). The device is an immunochromatographic test for the qualitative detection of IgM and IgG antibodies to SARS-CoV-2 nucleocapsid protein. It is unaffected by currently available vaccines which stimulate antibodies to the spike protein. The test manufacturers reported a sensitivity of 98.81% (95% CI: 97.25%, 99.61%), and a specificity of 98.02% (95%CI: 97.05%,98.74%). Evaluation studies also showed a combined sensitivity of 100.0% (95% CI: 92.9% –100%) and specificity of 98.0% (95% CI: 86.3% –99.7%) for SARS-COV-2

IgG/IGM.[17] The test was performed according to the manufacturer's instructions by placing 10 microlitres of serum into the sample port and then adding dilution buffer. The results were read after 15 minutes, and interpreted according to the manufacturer's recommendation.

Data Analysis

Data were managed using Microsoft Excel and subsequently analysed with IBM SPSS version 25.0. Descriptive statistics, including proportions, were calculated to summarize demographic and blood group data among participants. Seroprevalence rates of SARS-CoV-2 antibodies were stratified by age group and sex. Bivariate analyses were performed to test associations between SARS-CoV-2 antibody seropositivity and donor characteristics, including age, sex, and ABO blood group. The significance threshold was set at $p<0.05$, with the chi-square test applied to evaluate categorical variables.

RESULTS

Of the 274 blood donors, the majority, 227 (82.8%) were males and most, 123 (44.9%) were in the 21-30-year age bracket (Table 1). Figure 1 shows the distribution of blood donors with antibodies against SARS-CoV-2. Overall, 90 (32.8%) were positive for IgG; 75 (27.4%) were positive for IgM while 34 (12.4%) were positive for both IgG and IgM.

Table 1: Donor characteristics and association with SARS-CoV-2 seropositivity

Donor Characteristic	Donors (n=274) Frequency (%)	IgM +ve Frequency (%)	p-value	IgG +ve Frequency (%)	p-value
Age group (years)					
18-20	4 (8.8)	4 (17.4)		8 (34.8)	
21-30	123 (44.9)	39 (31.7)	0.181	44 (35.8)	0.701
31-40	74 (27.0)	22 (29.7)		20 (27.0)	
41-50	43 (15.7)	10 (23.3)		14 (32.6)	
>50	10 (3.6)	0 (0.0)		4 (44.4)	
Sex					
Male	227 (82.8)	62 (27.3)	0.961	75 (33.0)	0.881
Female	47 (17.2)	13 (37.7)		15 (31.9)	
ABO Blood group					
A	36 (13.1)	17 (47.2)	0.036	12 (33.3)	0.261
B	21 (7.7)	4 (19.0)		5 (23.8)	
AB	4 (1.5)	1 (25.0)		3 (75.0)	
O	213 (77.7)	53 (24.9)		70 (32.9)	
Rh Blood group					
Rh +ve	262 (95.6)	71 (27.1)	0.636	86 (32.8)	0.971
Rh-ve	12 (4.4)	4 (33.3)		4 (33.3)	

+ve= positive; -ve= negative; Rh =Rhesus

There was no significant association between seropositivity and either of age group, sex or Rhesus blood group. However, blood group A donors had a higher IgM seroprevalence than other blood groups and the association between IgG seropositivity and ABO blood group was significant (Table 1; p= 0.036).

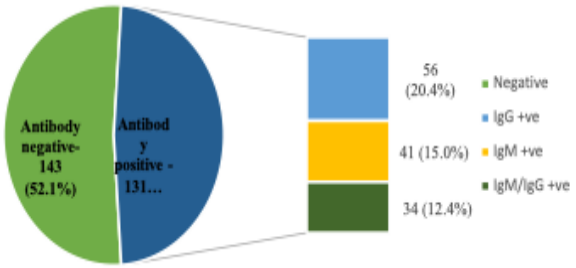

Figure 1: Distribution of SARS-COV-2 Antibodies in Blood Donors

DISCUSSION

Following the expiration of the COVID-19 PHEIC, there has been declining SARS-CoV-2 testing and limited surveillance data in Nigeria. Our study, conducted among blood donors in Benin City, Edo State between January and April 2024, provides valuable insights into the prevalence of natural immunity in this population, with close to a third testing positive for IgG antibodies against SARS-CoV-2 nucleocapsid protein. Nucleocapsid-specific antibodies serve as markers of natural infection and are absent in vaccine-induced immunity.[18] Given the transient nature of nucleocapsid-specific IgG, which are often undetectable within a year post-infection,[19] this figure possibly represents infections acquired over the preceding year and establishes a reference point for tracking post-pandemic immunity trends.

Serosurveillance using blood donors has proven invaluable in understanding SARS-CoV-2 transmission dynamics across various populations.[7-10] However, in Nigeria, such studies are sparse, with only one prior report from August 2020 showing IgM and IgG prevalence rates of 41% and 42%, respectively.[20] The present study, with an IgM prevalence of 27.4% and IgG prevalence of 32.8%, highlights the sustained value of donor-based serosurveillance for evaluating ongoing community transmission, especially in a context of decreased diagnostic testing. Replicating the study on a national scale could highlight post-pandemic COVID-19 epidemiologic trends for targeted public health interventions.

Despite being among the top ten states in Nigeria with the most reports of COVID-19,[13] only a limited number of seroprevalence studies have been conducted in Edo state. Comparing our findings to earlier seroprevalence reports, we observed a notable reduction in antibody levels. A nationwide sero-epidemiological survey conducted between June and August 2021 across 12 Nigerian states, including Edo, indicated a much higher seroprevalence of 74%.[16] Similarly, a cross-sectional survey among university students in Edo in 2022 reported a seroprevalence of 79% (Elimian et al., data under peer review). The relatively lower seropositivity in the present study may

be indicative of waning antibody levels, declining community transmission rates, or a combination of these factors. Nevertheless, the presence of IgM antibodies, a marker of recent infection, implies that transmission continues to occur, albeit, at a reduced rate.

Our study found no significant difference in SARS-CoV-2 seropositivity between sexes, aligning with findings from other national and global studies that suggest relative homogeneity in antibody prevalence across this demographic category.[16, 21-23] In addition, although individuals over 50 years of age exhibited slightly higher IgG seroprevalence, this trend was not statistically significant in our study. In contrast, earlier studies in Nigeria documented significant age-based associations with SARS-CoV-2 seroprevalence.[16, 22, 23] These were, however, community-based studies that included children and found significantly higher seropositivity in older age groups compared to children. This suggests that age-related trends may be context-dependent and influenced by sample composition.

We identified a remarkable association between ABO blood group and IgM seropositivity, with blood group A donors showing a significantly higher IgM prevalence than other blood groups. This finding corroborates other research, including a systematic review and meta-analysis, which indicate that blood group A individuals have a significantly higher risk of SARS-CoV-2 infection, a relationship initially reported in a large Chinese cohort.[24, 25] Moreover, Wu et al. recently demonstrated that SARS-CoV-2 variants, including Delta and Omicron, show a preference for binding to receptors on blood group A cells, suggesting a mechanism for this observed susceptibility.[26] Nonetheless, other study reports are conflicting and some even inconclusive about the associations between blood group and COVID-19 susceptibility and severity.[27] Further studies are therefore needed to explore this potential association and its implications for COVID-19 prevention strategies.

Despite the critical insights which this study provides, it has some notable limitations. As blood donors are generally healthier than the broader population, a selection bias is possible, potentially underestimating the true seroprevalence among the general public. Additionally, the study sample consisted predominantly of males, with females representing less than 20% of donors, which may limit the generalisability of the findings with respect to sex. The representativeness of the study is further constrained by its urban setting and exclusion of individuals younger than 18 years. Finally, lateral flow assays are generally considered to be less accurate than laboratory-based tests like enzyme-linked immunosorbent assays and chemiluminescence immunoassays. We minimised the impact of this limitation by using one of the most widely validated and best performing commercially available lateral flow tests.[17] The limitations notwithstanding, a key strength of our study was the use of a nucleocapsid-specific antibody assay, which measures natural infection-derived immunity, excluding the potential confounding effects of vaccine-induced antibodies.

CONCLUSION

In conclusion, our study demonstrated a relatively reduced but substantial level of SARS-CoV-2 antibodies among blood donors in Edo State, Nigeria as of early 2024, with blood group A individuals showing higher rates of recent infection. Continued monitoring of seroprevalence among blood donors can serve as a valuable public health strategy for tracking SARS-CoV-2 exposure trends and navigating surveillance in the evolving post-pandemic landscape.

Funding Sources**:** No funds were received for this study.

Conflict of Interest**:** The authors declare no conflicting interests.

Acknowledgements**:** The authors are grateful to Dr Kelly Elimian of the Department of Global Public Health, Karolinska Institute, Sweden for donating the test kits used in this study. We thank Gift Samuel for her assistance with laboratory testing.

Author Contributions: I.I.O and E.X.I contributed to conceptualization. I. I. O. and E.X.I contributed to the methodology. D.O, E.I and M.I.E conducted tests and collected the data. E. N. E., E.O.O and I.I.O performed the data analysis and data visualization. I. I. O. and E.O.O drafted the manuscript, which was edited by all authors. All authors have read and agreed with the submitted version of the manuscript. I. I. O. takes responsibility for the integrity of the work as a whole.

Data Availability: Data used for this study will be made available by the corresponding author upon reasonable request.

REFERENCES

1. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020; 382(8):727-733. doi: 10.1056/NEJMoa2001017
2. Eurosurveillance editorial team. Note from the editors: World Health Organization declares novel coronavirus (2019-nCoV) sixth public health emergency of international concern. Euro Surveill. 2020; 25(5):200131e. doi: 10.2807/1560-7917.ES.2020.25.5.200131e.
3. Cucinotta D, Vanelli M. WHO declares COVID-19 a pandemic. Acta Biomed. 2020; 91(1):157-160. doi: 10.23750/abm. v91i1.9397.

4. Peeling RW, Olliaro PL, Boeras DI, Fongwen N. Scaling up COVID-19 rapid antigen tests: promises and challenges. Lancet Infect Dis. 2021; 21(9):e290-e295. doi: 10.1016/S1473-3099(21)00048-7.
5. Lau H, Khosrawipour T, Kocbach P, Ichii H, Bania J, Khosrawipour V. Evaluating the massive underreporting and undertesting of COVID-19 cases in multiple global epicenters. Pulmonology. 2021; 27(2):110-115. doi: 10.1016/j.pulmoe.2020.05.015
6. Public Health Collaborators on Serosurveillance for Pandemic Preparedness and Response PHSeroPPR. Learning from serosurveillance for SARS-CoV-2 to inform pandemic preparedness and response. Lancet. 2023; 402(10399):356-358. doi: 10.1016/S0140-6736(23)00964-9.
7. Bloch EM, Kyeyune D, White JL, Ddungu H, Ashokkumar S, Habtehyimer F et al. SARS-CoV-2 seroprevalence among blood donors in Uganda: 2019-2022. Transfusion. 2023; 63(7):1354-1365. doi: 10.1111/trf.17449.
8. Adetifa IMO, Uyoga S, Gitonga JN, Mugo D, Otiende M, Nyagwange J et al. Temporal trends of SARS-CoV-2 seroprevalence during the first wave of the COVID-19 epidemic in Kenya. Nat Commun. 2021; 12(1):3966. doi: 10.1038/s41467-021-24062-3.
9. Stone M, Di Germanio C, Wright DJ, Sulaeman H, Dave H, Fink RV et al. Use of US blood donors for national serosurveillance of Severe Acute Respiratory Syndrome Coronavirus 2 antibodies: Basis for an expanded national donor serosurveillance program. Clin Infect Dis. 2022; 74(5):871-881. doi: 10.1093/cid/ciab537.
10. Kinoshita R, Arashiro T, Kitamura N, Arai S, Takahashi K, Suzuki T et al. Infection-induced SARS-CoV-2 seroprevalence among blood donors, Japan, 2022. Emerg Infect Dis. 2023; 29(9):1868-1871. doi: 10.3201/eid2909.230365.
11. World Health Organisation. Statement on the fifteenth meeting of the IHR (2005) Emergency Committee on the COVID-19 pandemic. 2023. Available at https://www.who.int/news/item/05-05-2023-statement-on-the-fifteenth-meeting-of-the-international-health-regulations-(2005)-emergency-committee-regarding-the-coronavirus-disease-(covid-19)-pandemic (Accessed on January 12, 2024)
12. Nigeria Centre for Disease Control. Official Statement to the news on COVID-19 Resurgence in Benue State. 2023. Available at https://ncdc.gov.ng/news/505/the-nigeria-centre-for-disease-control-and-prevention-launches-its-5-year-%282023-2027%29-strategic-plan (Accessed January 12, 2024)
13. Nigeria Centre for Disease Control COVID-19 situation report. Bi-weekly epidemiological report 22 Epi Week 7 – 8: 13th-26th February 2023. Available at https://ncdc.gov.ng/diseases/sitreps/?cat=14&name=An%20update%20of%20COVID-19%20outbreak%20in%20Nigeria (Accessed on February 23, 2024)
14. Obaseki DE, Osaigbovo II, Ogboghodo EO, Adeleye O, Akoria OA, Oko-Oboh GA et al. Preparedness and response of a tertiary hospital to the COVID-19 pandemic in Nigeria: challenges, opportunities and lessons. Trans R Soc Trop Med Hyg. 2021; 115(7):727-730. doi: 10.1093/trstmh/trab028.
15. Osaigbovo II, Igbarumah IO, Obaseki DE. Instituting coronavirus disease 2019 testing: opportunities and challenges of molecular laboratory diagnosis in a Southern Nigerian teaching hospital. Int Health. 2024; 13:ihae023. doi: 10.1093/inthealth/ihae023.
16. Kolawole OM, Tomori O, Agbonlahor D, Ekanem E, Bakare R, Abdulsalam N et al. SARS CoV-2 seroprevalence in selected states of high and low disease burden in Nigeria. JAMA Netw Open. 2022; 5(10): e2236053. doi: 10.1001/jamanetworkopen.2022.36053
17. Lutalo T, Nalumansi A, Olara D, Kayiwa J, Ogwang B, Odwilo E et al. Evaluation of the performance of 25 SARS-CoV-2 serological rapid diagnostic tests using a reference panel of plasma specimens at the Uganda Virus Research Institute. Int J Infect Dis. 2021; 112:281-287. doi: 10.1016/j.ijid.2021.09.020.
18. Jarlhelt I, Pérez-Alós L, Bayarri-Olmos R, Hansen CB, Petersen MS, Weihe P et al. Distinguishing SARS-CoV-2 infection and vaccine responses up to 18 months post-infection using nucleocapsid protein and receptor-binding domain antibodies. Microbiol Spectr. 2023; 11(5): e0179623. doi: 10.1128/spectrum.01796-23.
19. Krutikov M, Palmer T, Tut G, Fuller C, Azmi B, Giddings R et al. Prevalence and duration of detectable SARS-CoV-2 nucleocapsid antibodies in staff and residents of long-term care facilities over the first year of the pandemic (VIVALDI study): prospective cohort study in England. Lancet Healthy Longev. 2022; 3(1): e13-e21. doi: 10.1016/S2666-7568(21)00282-8.
20. Ifeorah I, Nna E, Okeke U, Ivo E, Ayemoba O, Yunusa T et al. Seropravelence of SARS CoV-2 IgM and IgG antibodies amongst blood donors in Nigeria. 2021; Preprint (Version 1) available at Research Square https://doi.org/10.21203/rs.3.rs-151037/v1
21. Patel M, Nair M, Pirozzoli E, Cienfuegos MC, Aitken E. Prevalence and socio-demographic factors of SARS-CoV-2 antibody in multi-ethnic healthcare workers. Clin Med (Lond). 2021; 21(1):e5-e8. doi: 10.7861/clinmed.2020-0619
22. Olaleye DO, Aminu M, Olusola BA, Segun TO, Faneye AO, Opayele AV et al. High seroprevalence of SARS-COV-2 antibody before Covid-19 vaccination in Nigerian communities. Int Arch Public Health Community Med 2022; 6 (3):083. doi.org/10.23937/2643-4512/1710083
23. Audu RA, Stafford KA, Steinhardt L, Musa ZA, Iriemenam N, Ilori E et al. Seroprevalence of SARS-CoV-2 in four states of Nigeria in October 2020: A population-based household survey. PLOS Glob Public Health. 2022; 2(6):e0000363. doi: 10.1371/journal.pgph.0000363.
24. Kabrah SM, Kabrah AM, Flemban AF, Abuzerr S. Systematic review and meta-analysis of the susceptibility of ABO blood group to COVID-19 infection. Transfus Apher Sci. 2021; 60(4):103169. doi: 10.1016/j.transci.2021.103169.
25. Zhao J, Yang Y, Huang H, Li D, Gu D, Lu X et al. Relationship between the ABO blood group and the coronavirus disease 2019 (COVID-19) susceptibility. Clin Infect Dis. 2021; 73(2):328-331. doi: 10.1093/cid/ciaa1150.
26. Wu SC, Arthur CM, Jan HM, Garcia-Beltran WF, Patel KR, Rathgeber MF et al. Blood group A enhances SARS-CoV-2 infection. Blood. 2023; 142(8):742-747. doi: 10.1182/blood.2022018903
27. Gutiérrez-Valencia M, Leache L, Librero J, Jericó C, Enguita Germán M, García-Erce JA. ABO blood group and risk of COVID-19 infection and complications: A systematic review and meta-analysis. Transfusion. 2022; 62(2):493-505. doi: 10.1111/trf.16748.