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COVID-19 in Blood Donors at Laquintinie Hospital in Douala during the Third Wave: A Cross Sectional Study

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Abstract

This study was conducted at Laquintinie Hospital during the period between September 2021 and April 2022. The total number of cases who came to donate blood was 150 donors aged 18 to 60 years; 48 were excluded for several reasons. Several examinations were conducted for participants that were accepted for a study (n = 102 [2 (2.0%) were women and 100 (98.0%) men]), the prevalence of SARS-CoV-2 in nasopharyngeal samples was 11.8%. The mean CD4 count was 763.23 ± 194.61 cells/µl with endpoints [250 - 1400] cells/µl. IgG antibodies were present in 62.75% of cases. No statistically significant relation was found between SARS-CoV-2 carriage and IgG level or CD4 level (p = 0.850 & 0.056). Concerning the blood group, 57.3% (58) of the donors were of blood group O Rhesus positive; 19.4% (20) of blood type A Rhesus positive; and 2.9% were of blood group A Rhesus negative. Pupils and students represented 35.3% of our population, followed by employees at 25.5%. The SARS-CoV-2 positivity rate was 11.8% (n = 12). The transfusion transmitted infections (TTI) rate was 12.8% with 1.2% (1) positive HIV serologies, 5.8% (6) positive for HBsAg, 3.9% (4) for HCVAb, and 1.9% (2) positive TPA.

Keywords

SARS-COV-2, COVID-19, IgG, CD4

1. Introduction

Coronavirus disease 2019 (COVID-19) is a highly contagious viral illness caused

by a Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), causing over 3.8 million deaths around the world [1]. Since COVID-19 erupted in Wuhan, Hubei Province, China in late December 2019, SARS-CoV-2 quickly spreads across the world, causing the World Health Organization (WHO) to declare it a global pandemic on March 11, 2020, but the source/origin of the COVID-19 was still controversial. Since being declared a global pandemic, COVID-19 has devastated many countries around the world, rendering inaccessible hospitals [1]. The pandemic also had a negative impact on blood stocks due to the decrease in blood donations and the reduced availability of blood collection centers [2] [3] [4] [5] [6].

On February 17 2021, the World Health Organization (WHO) reported no cases of transmission of SARS-CoV-2 through transfusion of blood and its constituents [7]. Other reports published indicate that, despite the detection of viral Ribonucleic Acid (RNA) in some cases, labile blood products collected from donors during the pre-symptomatic phase of SARS-CoV-2 infection did not transmit infection [8]. Therefore, the risk of transmission of SARS-CoV-2 through the transfusion of blood collected from asymptomatic people is theoretical [9]. However, blood donation from confirmed cases of COVID-19 or people who have had recent contact with a known infected person should not be accepted [10] [11]. Screening of blood stocks is premature in the absence of cases of transmission by transfusion or proven infectivity of SARS-CoV-2 in blood collected from asymptomatic people, including pre-symptomatic people [12]. On the other hand, COVID 19 might have an effect on transfusion through passive immunity, which can protect blood recipients against coronavirus. Infection with SARS-CoV-2 activates innate and adaptive immune responses, including the induction of virus-specific T and B cells, but dysfunctional immune responses, such as inflammatory cytokine storms, are probably associated with the severity of COVID-19 [13]. CD4 T cells play essential roles in coordinating immune responses via the help of B cells for Antibody production. They also promote effector activity of CD8 T cells and the establishment of B and T cell memory [14]. SARS-CoV-2-specific CD4 T cells produce IL-2 and IFN-γ, suggesting that COVID-19-recovered individuals exhibit a TH1 cell response [15] [16] [17].

The purpose of this study was to evaluate the risk of SARS-CoV-2 carriage in the blood donor, the immune status by the prevalence of antibodies, CD4 level and the relation between SARS-CoV-2 carriage and immune status, likely to have an impact on blood transfusion.

2. Methods and Materials

2.1. Study Design and Setting Population

We carried out a cross-sectional prospective study carried out from September 2021 to April 2022 (8 months).

The study subjects were individuals who came to donate blood at Laquintinie Hospital, only those who were eligible to blood donation were included in our

study. We used a consent form, a medical questionnaire and a medical examination to include participants.

2.2. Inclusion and Exclusion Criteria

Candidates were received in the blood bank during the study; consenting volunteers were selected. Inclusion criteria of participants to the study: those who were eligible to blood donation were included in the study (subjects aged 18 to 60 years, body weight ≥ 50 kg, with Hemoglobin level ≥ 13 g/dl for men and ≥12 g/dl for women; with no concomitant diseases, no pregnancy, and no breastfeeding). Based on the above criteria, donors were recruited. Those who were not able to be give blood were excluded (difficulties to puncture vein, shock during blood donation).

2.3. Sampling Procedure

The sample size was calculated based on the formula for basic sample size calculation for random sampling [18]. The 95% confidence level and 6% prevalence of COVID-19 in Cameroon were used [19] and 87 participants were required for the minimum sample size. The participants were recruited at the blood bank using convenience sampling method. During the period of the study 150 candidates were received in the blood bank; sociodemographic characteristics (age, gender, occupation) of the participants were assessed by self-report and a face-to-face interview, the candidate then followed the medical selection conducted by well trained personnel from the blood bank team. After the medical examination 48 did not met the criteria, were not able for donation, or refused to participate in the study forming an exclusion rate of 32%, 102 candidates were included and their samples were collected to assessed biological analysis such as ABO and Rhesus blood group; Human Immunodeficiency Virus (HIV), Antibodies against Hepatitis C Virus (HCVAb), Hepatitis B Surface Antigen (HBsAg) and Treponema Pallidum Assay (TPA) for the determination of TTI; the immune status evaluated by Cluster of Differentiation 4 (CD4) count and IgG testing; and the carriage of SARS-CoV-2 Ribonucleic Acid (RNA).

2.4. Immune Status Assessment

2.4.1. CD4 Count

The sample was collected into labelled tri-potassium ethylene diamine tetra-acetic acid tubes EDTA tube (5 ml) mixed gently and immediately tested or store at 2˚C - 8˚C for 24 hours; the CD4 count was done by automated technique by cytometry. An aliquot of an EDTA whole blood sample is mixed with the antibody (CD4-PE mAb) conjugated to the fluorochrome in a 1:1 ratio. The procedure consists of adding 20 mm^3 of whole blood into reagent tube followed by gently mixing and incubation at room temperature in the dark for 15 minutes. The buffer is added and the sample is ready for analysis on a CyFlow Counter® flow cytometer (Am Flugplatz 13, Germany). The light source excites the fluorescent

dye binds to the stained cell and the emitted light is detected as a precise volume of blood sample passes through the instrument. The integrated software calculates the concentration of the specific cell population. The normal level of CD4 was [500 - 1500]/µl.

2.4.2. IgG Testing

A volume of 5 ml was collected in a dry tube, the serum was obtained after centrifugation of dry tube at 1500 rates/minutes the supernatant was collected immediately tested or and stored at −20˚C before the analysis. The analysis was done automatically using the Enzyme Linked Fluorescent Assay (ELFA) technique on Vidas® (Marcy-l'Etoile, France), 100 µL of sample was added to the cartridge. The test is based on a final reading in fluorescence and allows qualitative results to be obtained. This test allows the semi-quantitative detection of the level of IgG antibodies directed against the receptor-binding domain (RBD—Receptor Binding Domain) of the viral protein Spike (S) a specificity of 99.9% and a sensitivity of 88.6% (Number of days after positive Polymerase Chain Reaction (PCR) 8 - 15 days) and 96.6% (Number of days after positive PCR ≥ 16 days). The result is automatically calculated according to a Standard (S1), the fluorescence of the standard is compared to the fluorescence of the sample (index = fluorescence of sample/fluorescence of S1). The results are interpreted as follow: index < 1 is considered Negative (absence of SARS-CoV-2 IgG), an index ≥ 1 is considered Positive (presence of SARS-CoV-2 IgG).

2.5. Carriage of SARS-CoV 2 RNA

A nasopharyngeal swab was collected into one milliliter (1 ml) viral transport medium (VTM).

The test was done by PCR for SARS-CoV-2 using the DaAn gene protocol (Guangzhou, Guangdong, China).

Virus inactivation and RNA Extraction: the total nucleic acid from the nasopharyngeal swabs in VTM was extracted using a DaAn Gene nucleic acid extraction kit (DaAn Gene Co, Ltd., of Sun Yat-sen University, China) as per manufacturer's instructions. The extracted ARN was stored at −20˚C awaiting SARS-CoV-2 RT-PCR.

Amplification: the SARS-CoV-2 RNA detectionkit of DaAn Gene 2019-nCoV detection kit (DaAn Gene Co, Ltd., of Sun Yat-sen University, China) was used: The primer and probe set are designed to detect ORF1ab and nucleo-capsid protein (N) gene sequences from the SARS-CoV-2. Human housekeeping gene Ribonuclease P (RNP) was developed as the target gene for the internal control for monitoring the specimen collection, nucleic acid extraction process and PCR amplification process. The probe detection modes were set as: ORF1ab: VIC, Quencher: NONE, N-Gene: FAM, Quencher: NONE, Internal Control: Cy5, Quencher: NONE, Passive reference: NONE. The cycle threshold (Ct) was set according to laboratory verification and determination of the appropriate Ct for the target genes. The result was considered positive or negative.

2.6. Ethical Consideration

This study was approved by the Ethical committee of the Faculty of Medicine and Pharmacy of The University of Douala N˚2983 CEI-Udo/04/2022/M.

2.7. Statistical Analysis

Data were analyzed using Statistical Package for Social Sciences version 20.0 (SPSS 20.0). Categorical variables were presented as frequencies and percentages. Continuous variables were presented as means and standard deviation. The strength of association among PCR result and sociodemographic data; PCR results, IgG and CD4 count was assessed using a bivariate analysis by logistic regression or chi square, the degree of association was expressed by odds ratio and the confidence interval was set at 95%. The level of statistical significance was set at a p-value of <0.05.

3. Results

3.1. Description of the Study Population

Sociodemographic characteristic of the study population is recorded in Table 1. On the 102 participants recruited, 2 (2.0%) were women and 100 (98.0%) men, the sex ratio was 50. The age of the participants varied between 18 and 28 years with an average age of 30.94 ± 8.42 years; the modal class being [18 - 28] years with 44cases (43.1%) Concerning the blood group, 57.3% (58) of the donors, were of blood group O Rhesus Positive; 19.4% (20) of blood type A Rhesus Positive; 2.9% were of blood group A Rhesus negative. Pupils/students represented 35.3% of our population, followed by employees 25.5%. The SARS-CoV-2 positivity rate was 11.8% (n = 12). The TTI rate was 12.8% (Table 1) with 1.2% (1) positive HIV serologies; 5.8% (6) positive for HBsAg, 3.9% (4) for HCVAb and 1.9% (2) positive TPA.

The donor serum analysis showed that 62.8% (64) of donors had SARS-CoV-2 IgG antibodies (presence of antibodies: positive ≥ 1) (Table 1).

The analysis of whole blood from blood donors showed that 99.0% of donors had a normal CD4 count [500 - 1500] cells/ul. The average CD4 count in the study donors was 763.23 ± 194.61 cells/µl with endpoints [250 - 1400] cells/µl.

3.2. SARS-CoV-2 Carriage Immune Status and Associated Factors

3.2.1. Repartition of SARS-CoV-2 Carriage According to Sociodemographic and Para-Clinical Characteristics

The results for the association between sociodemographic characteristics, paraclinical and SARS-CoV-2 carriage are reported in (Table 2). It was found that all positive donors were males (n = 12). The carriage of SARS-CoV-2 was most prevalent in Young donors aged [18 - 28] and [28 - 38] years old (respectively OR = 3.55×10^8; 95% CI: 4.03×10^9 - 3.21×10^7; p = 0.000 and OR = 2.14×10^8; 95% CI: 2.15×10^9 - 2.13×10^7, p = 0.000). With occupation, the result showed that pupils/students, shopkeeper and employee are more likely to have a nega-

tive PCR result than unemployee (respectively OR = 0.68; 95% CI: 0.06 - 7.14; p = 0.754. OR = 0.22; 95% CI: 0.02 - 2.13; p = 0.194. OR = 0.47; 95% CI: 0.04 - 5.03; p = 0.540). On the other hand, blood group A positive is more associated with negative PCR than blood group O positive (OR = 1.44; 95% CI: 0.27 - 7.42; p = 0.663); on the same hand blood group B positive is more associated with negative PCR than blood group O positive (OR = 1.2; 95% CI: 0.24 - 6.65; p = 0.769), the absence of TTI was more related to positive PCR than the presence of TTI (OR = 1.47; 95% CI: 0.21 - 10.38). The risk of having a negative PCR in IgG presence was 1.08 (CI: 0.56 - 1.81; p = 0.06), 05/38 (13.1%) of donors had a positive PCR in the absence of antibodies. The risk of having a negative PCR with CD4 ≥ 500 C/µl was 1.09 (95% CI: 0.92 - 1.29; p = 0.738).

Table 1. Characteristics of blood donors.

Variable	Modality	Number N = 102 (n)	Percentage (%)
Gender	Male	100	98
	Female	02	2
Age range (years)	[18 - 28]	44	43.1
	[28 - 38]	38	37.3
	[38 - 48]	17	16.7
	[48 - 58]	03	2.9
Occupation	Pupils/Students	26	25.4
	Shopkeeper	23	22.6
	Employee	36	35.3
	Unemployee	17	16.7
Blood Group	A negative	03	2.9
	A positive	20	19.4
	AB positive	03	2.9
	B positive	18	17.5
	O positive	58	57.3
TTI	Yes	13	12.8
	No	89	87.2
SARS-CoV-2 RNA carriage	Positive PCR	12	11.8
	Negative PCR	90	87.2
IgG testing	Presence of IgG	38	37.2
	Absence of IgG	64	62.8
CD4 count	Level of CD4 < 500 C/µl	1	1.2
	Level of CD4 ≥ 500 C/µl	101	98.8

SARS-CoV-2: Severe Acute Respiratory Syndrome Corona Virus 2; PCR: Polymerase Chain Reaction; TTI: Transfusion Transmitted Infection; IgG: Immunoglobulin G; absence of IgG: index < 1; presence of IgG: index ≥ 1.

Table 2. Distribution of SARS-CoV-2 carriage according to sociodemographic and paraclinical characteristics.

Variable	Modality	Number N = 102 n (%)	Negative PCR N = 90 n (%)	Positive PCR (ref) N = 12 n (%)	OR (CI 95%)	p
Gender	Male	100 (98.0)	88 (88.0)	12 (12.0)	0.97 (0.94 - 1.00)	1
	Female	02 (2.0)	02 (100)	-	Ref	
Age range (years)	[18 - 28]	44 (43.1)	38 (86.4)	06 (13.6)	3.55×10^8 (4.03×10^9 - 3.21×10^7)	0.000
	[28 - 38]	38 (37.3)	33 (86.9)	05 (13.1)	2.14×10^8 (2.15×10^9 - 2.13×10^7)	0.000
	[38 - 48]	17 (16.7)	16 (94.1)	01 (5.9)	6.32×10^8 (6.32×10^8 - 6.32×10^8)	
	[48 - 58]	03 (2.9)	03 (100)	-	Ref	
Occupation	Pupils/Students	36 (35.3)	33 (91.7)	03 (8.3)	0.68 (0.06 - 7.14)	0.754
	Shopkeeper	23 (22.6)	18 (78.3)	05 (21.7)	0.22 (0.02 - 2.13)	0.194
	Employee	26 (25.4)	23 (88.5)	03 (11.5)	0.47 (0.04 - 5.03)	0.540
	Unemployee	17 (16.7)	16 (94.1)	01 (5.9)	Ref	
Blood Group	A negative	03 (2.9)	03 (100)	-	9.62×10^7 (9.62×10^7 - 9.62×10^7)	0.432
	A positive	20 (19.4)	18 (90.0)	02 (10.0)	1.44 (0.27 - 7.42)	0.663
	AB positive	03 (2.9)	03 (100)	-	9.62×10^7 (9.62×10^7 - 9.62×10^7)	-
	B positive	18 (17.5)	16 (88.9)	02 (11.1)	1.2 (0.24 - 6.65)	0.769
	O positive	58 (57.3)	50 (86.2)	08 (13.8)	Ref	
TTI	Yes	13 (12.8)	13 (100)	-	Ref	0.696
	No	89 (87.2)	77 (86.5)	12 (13.5)	1.47 (0.21 - 10.38)	
IgG testing	Absence of IgG antibodies	38 (37.2)	33 (86.9)	05 (13.1)	Ref	0.758
	Presence of IgG antibodies	64 (62.8)	57 (89.1)	07 (10.9)	1.08 (0.56 - 1.81)	
CD4 count	Level of CD4 < 500 C/µl	01 (1.0)	-	01 (100)	Ref	0.118
	Level of CD4 ≥ 500 C/µl	101 (99.0)	90 (89.1)	11 (10.9)	1.09 (0.92 - 1.29)	

PCR: Polymerase Chain Reaction; Ref: Reference; TTI: Transfusion Transmitted Infection. PCR: Polymerase Chain Reaction; OR: Odds Ratio; IgG: Immunoglobulin G; CD4: Cluster of differentiation 4; CI: Confidence Interval; absence of IgG: index < 1 presence of IgG: index ≥ 1.

3.2.2. Distribution of IgG Antibodies According to CD4 Count

Regarding the distribution of IgG antibodies all participants presenting absence of antibodies had a normal level of CD4, 1/64 (1.6%) presented a low level of CD4. The relation between the level of CD4 and IgG was not statistically significant (p = 1) with an OR = 1.60 (95% CI = 1.37 - 1.86) (Table 3).

Table 3. Distribution of IgG antibodies according to CD4 count.

Variable	Number N = 102 N (%)	Level of CD4 < 500 C/µl N = 1 (1.0%) N (%)	Level of CD4 ≥ 500 C/µl N = 101 (99.0%) N (%)	OR (CI-95%)	P
Absence of IgG antibodies	38 (37.3)	-	38 (100)	Ref	1
Presence of IgG antibodies	64 (62.8)	01 (1.6)	63 (98.4)	1.60 (1.37 - 1.86)	

P-value < 0.05: Significant; OR: Odds Ratio; IgG: Immunoglobulin G; CI: Confidence Interval; CD4: Cluster of differentiation 4; Presence of antibodies: index ≥ 1; Absence of antibodies: index < 1.

4. Discussion

We conducted an analytical cross-sectional study at the blood bank of the Laquintinie hospital in Douala. A total of 102 blood donors were included in the study with a male predominance of 98.0% and a sex ratio of 50; similar result was found by Fohoue *et al.* at Yaoundé Central Hospital in 2016; they demonstrated that there is generally a predominance of the male gender (93.4%) with a sex ratio varying from 3 to 5 men for one woman [20]. The age of our donors was between 18 and 58 years old with an average age of 30.94 ± 8.42 years similar to the result of Fohoue *et al.* which was 29 ± 8 years old with extremes of 18 and 58 years old [20]. The most represented age group in our study was that of [18 - 28] years, (43.1%). Regarding the professional status of the donors, the pupils/students were the most representative; this justifies the predominance of those with a higher level of education (35.3%). This observation was also made by Tagny *et al.* in 2013 usually, blood donor is young, with an average age of 26 years (17 - 60 years old) [21]. The distribution of blood group among blood donors showed that O rhesus positive blood group was dominant with a prevalence of 57.3% (n = 58) followed by A rhesus positive blood group (19.4%; n = 20) in agreement with the results of Hadj *et al.*, in their 133 COVID positive patients, O positive blood group was the most frequent (41.3%; n = 55) followed by the A positive blood group (33, 8%; n = 45) [22].

Regarding the biological data, the prevalence of SARS-CoV-2 was 11.8% and it was dominant in men. The high level of infected donors can be explained by the fact that most of our donors were recruited during the 3rd wave; our prevalence was higher than the global prevalence in Cameroon [23]. The prevalence obtained in our study was dominant in 2 age groups: [18 - 28] and [28 - 38]; however, there was no association between SARS-CoV-2 and age groups. Alix *et al.* in 2021 carried out a study on COVID-19 in Haute-Corse and obtained similar results with an age group most affected by SARS-CoV-2 of [10 - 29] years [24]. Shopkeepers represented the class of workers most affected because they are in direct contact with users and generally do not respect the barrier measures recommended by the WHO. The A rhesus positive group and B rhesus positive group were less contaminated than the O positive group, suggesting that certain blood groups are more exposed than others to being contaminated; this result is different from the one published by Zhao *et al.*, it was observed that individuals

from blood group O were under-represented [25]. The IgM anti-A and ant-B Immunoglobulin isotype present in A and B group is less protective than the IgG in the serum of individuals of blood group O [26], but in our study this seems to be not accepted probably due to geographical and genetic variation of each country. The frequency of distribution of IgG SARS-CoV-2 antibodies was 62.8% with a positivity threshold ≥3 IU/ml, this threshold was different from the 10 IU/ml used by Chalton *et al.* in 2021 in Canada and can explain the difference in prevalence obtained [27], some issues should be considered, including the appropriate test to assess seroprevalence and the threshold for identifying positive and negative samples. The higher rate of IgG positivity found among our donors comparing to the 6.4% for male and 4.7% for female donors in Minas Gerais by Chaves *et al.*, suggests that the period of the pandemic might change the prevalence and more persons has been previously exposed [28]. Our result was lower than the 97% reported by the Pasteur Institute in 2021 in France [29]. The distribution of SARS-CoV-2 carriage according to IgG antibody showed more cases of positive PCR in the absence of antibodies, possible explanation are: infected persons who do not develop clinical disease and may possibly combat the coronavirus on the mucosa of their upper respiratory tract preventing a systemic humoral immune response; the humoral immune response towards SARS-CoV-2 is dependent on the duration and magnitude of viral antigen exposure [30] [31] [32]. Yan *et al. in* 2021 in China did a study on the carriage of SARS-CoV-2 according to the concentration of IgG, they demonstrated that the concentration of IgG antibodies depends on the severity of COVID-19 disease and that there is no significant difference between IgG antibody concentration and SARS-CoV-2 carriage [33]. It is known that COVID-19 cause lymphopenia and the number of total T cells, CD4+ and CD8+ T cells dramatically reduced in COVID-19 patients, especially in patients requiring Intensive Care Unit (ICU) care [34], SARS-CoV-2 carriage could affect T cell population counts in the peripheral blood, most frequently with severe cases [35] [36]. In our study, the concentration of CD4 was normal in most of cases and showed no association to SARS-CoV-2 carriage; the absence of relation between SARS-CoV-2 carriage and CD4 count can be explained by the fact that blood donors are almost asymptomatic.; Grifoni *et al.* in 2021 in Germany demonstrated in accordance with our finding that there was no significant association between SARS-CoV-2 carriage, IgG antibody concentration and CD4 count [37].

A close connection between CD4+ T cells and antibody production in COVID-19 convalescent patients demonstrated by Fang *et al.* [38], but in our study there was also no relation between IgG level and CD4 count, probably due to the fact that donors are asymptomatic.

5. Limitations

During our study, we faced many difficulties which are as follows: the size of our sample and limited representation of females (only 2 females) do not allow us to

interpret our results at the national level, the nasopharyngeal swab is not pleasant hence refusals of participants; but our findings might be interesting for the recruitment of donors for the preparation of a convalescent plasma basis on the immune status and SARS-CoV-2 carriage.

6. Conclusion and Recommendations

From our study, it appears that the prevalence of SARS-CoV-2 carriage in donors is high (11.8%) compared to the general population. The CD4 count is normal, the majority of donors are positive to IgG with a threshold of 3 UI/ml; there is no significant association between SARS-CoV-2 carriage, IgG antibodies and CD4 count. We, therefore, recommend that more studies on SARS-CoV-2 carriage, the immune status of the blood donor and their effect on blood recipient should be done due to the virus mutation. We further recommend that other immunological analysis such as cytokine measurement and neutralizing antibodies should be assessed and their effect on recipient should be monitored.

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Consent for Publication

Not applicable.

Data Availability Statement

Data is available from the corresponding authors (C.I.M)

Authors' Contributions

C.I.M, S.A.L, initiated the project. C.I.M, S.A.L conducted biological tests. C.I.M, S.A.L, E.V.V, B.E.B conducted statistical analyzes. C.I.M., S.A.L, B.E.B, E.V.V, E.L.E, E.N.E wrote and corrected the manuscript. J.A.N supervised the study. All the authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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