Prevalence of Syphilis Infections Among Volunteer Blood Donors in Jinan Blood Center, China: A 15-Year Retrospective Study

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Background: Syphilis infections among volunteer blood donors increased rapidly in recent years. It is important to analyze the demographics of seropositive donor groups and help to recruit donors from low-risk population.

Objective: The aim of this study was to analyze the syphilis prevalence among volunteer blood donors in Jinan Blood Center and give direction to blood recruitment.

Methods and Materials: A cross-sectional study was conducted among blood donors in Jinan, China. Socio-demographic data and blood donation testing data from January 2007 to December 2021 were extracted from the database of blood management software of Jinan Blood Center for analysis. All blood samples were screened by ELISA, and those anti-TP-positive samples were counted and analyzed by sex, age, educational background, occupation and blood donation times. Logistic regression was used to explore risk factors associated with syphilis infection.

Results: Totally 700,757 blood samples were collected in the study during 2007 to 2021, 2290 cases were detected anti-TP positive with a positive rate of 0.33%. Female, 35–44 years old, with a lower education degree, farmers and first-time donors were the high-risk subgroups.

Conclusion: Consultation and identification of high-risk population groups should be improved. Measures should be taken to make the donor recruitment more professional and detailed.

Keywords: transfusion-transmitted infections, volunteer blood donors, syphilis, China

Introduction

Syphilis is an infectious disease caused by Treponema pallidum (T. pallidum). Unprotected sexual contact and transplacental spread during pregnancy are its main spreading ways.¹ It is one of the mandatory serologically tested transfusion-transmitted infections (TTI) recommended by the World Health Organization (WHO).² China launched the Blood Donation Law of the People’s Republic of China in 1998. Since then, paid donation was banned, and voluntary blood donation was implemented nationally.³ All blood specimens donated must go through laboratory tests of hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV) and Treponema pallidum (TP). Jinan, the capital city for Shandong province of China, has achieved 100% voluntary blood donations as well.

In China, syphilis, together with Severe Acute Respiratory Syndrome (SARS), Corona Virus Disease 2019 (COVID-19), hepatitis and Acquired Immune Deficiency Syndrome (AIDS), and other infectious diseases such as measles, poliomyelitis, pulmonary tuberculosis, etc, is classified as class B infectious disease.⁴ Class A infectious disease is pestis and cholera.⁴ Syphilis was once a venereal disease with high prevalence before 1949 in China. Surveillance studies in the 1950s showed that the infection was present in as many as 84% of prostitutes and 5% of the general population in some large cities, and 2–3% of rural residents.⁵⁶ After the establishment of the People’s Republic of China in 1949, the
government took a series of measures to eliminate syphilis, including banning brothels, syphilis screening in general population, free treatment, and so on. The effect was very significant. Syphilis was eradicated in the 1960s. However, since the late 1970s, with the implementation of reform and opening policy and the development of society, syphilis has revived, the number of cases as well as the incidence rate has increased significantly. In 1999, 80,406 cases were reported, with an annual incidence rate of 6.50/100,000. In 2009, 327,433 cases were reported, with an annual incidence rate of 24.66/100,000. The incidence rate increased by 14.3% annually. In 2009, the number of reported syphilis cases ran up to the third place among the reports of class A and B infectious diseases in China.

Jinan is the capital city of Shandong province with a population of 9 million. In recent years, the increasing liberal sexual behavior and the growing mobility with the economic development made sexually transmitted diseases (STDs) such as syphilis, AIDS, etc, spread rapidly. According to Yang’s investigation, in Jinan, the incidence of syphilis among the general population went up to 17.86/100,000 in 2018 from 9.29/100,000 in 2009, which was much higher than the average incidence of Shandong province over the same period. Totally, 10,301 cases of syphilis were reported from 2009 to 2018 with an overall prevalence of 114/100,000 in Jinan, among which 208 cases were congenital syphilis with an overall prevalence of 2.3/100,000. The incidence of congenital syphilis showed an obvious downward trend from 0.5/100,000 in 2011 to 0.13/100,000 in 2018.

Yang’s study also indicated that the ratio of male to female of syphilis cases was 1.12:1. The patients of the 20 to 49 years old group and over 60 years old group accounted for 64.24%. More than half (51.18%) of the patients had recessive syphilis. Male and 20–49 years old groups are major donation population. The high incidence of these two groups and the high ratio of recessive syphilis bring much more risks and challenges to transfusion safety. In China, syphilis gets less social concern and publicity from the society compared with HIV. So, people could not acquire enough knowledge including the dangerous behavior easily being affected, where they can get detection or a regular medical treatment of syphilis. In Jinan Blood Center, according to our annual statistics, the seropositive rate of anti-TP among blood donors showed a slowly upward trend before 2007. The incidence grew dramatically from 0.32% in 2007 to 0.56% in 2010 and kept in a high level for four years. Therefore, we trigger the protocol to conduct rapid screening of Treponema pallidum antibody before donation in 2014, the incidence decreased slowly and now maintains a level of about 0.25%; however, this is still much higher than the seropositive rates of serum markers of HBV, HCV and HIV. Although China carried out China’s syphilis prevention and control program (2010–2020) to control syphilis, the reported cases kept rising from 358,534 cases in 2010 to 535,819 cases in 2019. The cumulative incidence of syphilis was 430/100,000 among general population from 2007 to 2021 according to data opened by the government. Although the increased incidence may partially be due to the development of Electronic Direct Reporting System for Infectious Diseases and intensified screening among high-risk population, we cannot ignore the large number of infected individuals. And this is a huge risk for blood transfusion safety. So we think it is essential to identify the high-risk groups in local population to take measures to ensure transfusion safety.

Materials and Methods
Study Design, Area and Period
We designed a cross-sectional retrospective data study to analyze the risk factors of syphilis among blood donors from January 2007 to December 2021. Testing data and demographics of donors were extracted from the database of Jinan Blood Center. Crude prevalence of T. pallidum antibodies was calculated. Demographic data, including age, gender, maximum educational attainment and occupation, were routinely collected on donors. To verify the accuracy of the results of ELISA test, data of confirmation test by TPPA (Treponema pallidum particle agglutination assay) were collected and calculated. The study was conducted at Jinan Blood Center. Jinan Blood Center is located in Jinan, the capital city of Shandong Province in eastern China, which is 400 km away from Beijing, the capital of China.

Sample Size Determination
The sample size was calculated using a sample size calculation forum for multigroup proportions, and the following assumption was considered: $\alpha = 0.05$, $\beta = 0.2$, $\nu$ (degree of freedom) = group-1 (We took the maximum number of subgroups, group = 6), $\lambda = 12.83$ (looked up from table of \( \lambda \) value), $P_{max} = 0.008$, $P_{min} = 0.003$. 

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The predicted sample size was 5310 every group, and the total sample size needed in the study was no less than 31,860 (5310*6).

**Study Population and Blood Donation Procedure**

The sample technique was accidental sampling as the study population was volunteer blood donors recruited by Jinan Blood Center during January 2007 and December 2021. Only data from those who donated successfully and underwent regular laboratory tests were included in the study. Exclusion criteria: discontinued donation, confidential blood abandonment and those without laboratory test because of unqualified blood samples. Nine mobile vehicles and eight fixed-site donor rooms were dispatched around the city to make the blood donation convenient. Volunteers could go either of the sites to donate blood. Before donation, each potential donor should sign a donation registration form which is routinely collected on donors, including basic information such as age, gender, education, occupation, and a detailed health questionnaire. The questionnaire contains a series of disease or health status that could not donate blood temporarily or permanently. Permanent deferral includes 22 medical conditions such as a series of chronic diseases and infectious diseases (HIV, syphilis, etc.), transplant, systemic disease, cancer, important organ resection, etc. Temporary deferral includes 20 conditions with a deferral period from three days to three years. For example, one cannot give a donation if he had a cold or gastroenteritis within a week or had high-risk sexual behaviors, got inoculation of certain vaccines, and received blood product transfusions within a year. Women cannot donate blood during pregnancy and lactation and three days before and after menstruation.16

Then, rapid testing of haemoglobin would be taken according to national guidelines16 to prevent anaemia patients from donating and ensure blood quality. In Jinan Blood Center, rapid testing also includes blood type, hepatitis B surface antigen, alanine transaminase (ALT) and *Treponema pallidum* antibody (anti-TP). In China, pre-donation testing of hepatitis B surface antigen, alanine transaminase, is conducted because of the high prevalence of hepatitis for decades. And the rapid testing of anti-TP was also conducted before donation since 2014 because of the growing anti-TP positivity rate among blood donors in Jinan those years. Weight, blood pressure, heart rate and body temperature were also measured. The results of those met the criteria of Whole Blood and Component Donor Selection Requirements (GB 18467–2001, GB 18467–2011, China) could proceed to donate. All blood donors were volunteers and non-remunerated. The criteria for donor eligibility are as follows: (1) Age: 18–60 years old; (2) Weight: Male ≥50 kg, Female ≥45 kg; (3) Blood Pressure: 12.0 Kpa (90 mmHg) ≤ systolic pressure <18.7 Kpa (140 mmHg) and 8.0 Kpa (60 mmHg) ≤ diastolic pressure <12.0 Kpa (90 mmHg); (4) Hemoglobin levels: for males, greater than 120 g/L, and for females, greater than 115 g/L; (5) Rapid testing is negative and ALT ≤50IU.

**Sample Collection and Laboratory Screening**

During the donation procedure, 5 mL peripheral blood was taken with an EDTA anticoagulation sterile test tube. All the donor samples were tested for HBsAg, anti-HCV, anti-HIV (types 1 and 2) and syphilis, with enzyme linked immunosorbent assays (ELISA) kits. The testing process followed the Technical Operation Procedures in Blood Stations of China, according to which ELISA tests of blood donors should be carried out with two different equipment and two different test kits.16 The equipment of anti-TP testing was FAME24/20 (Hamilton, Switzerland) and Uranus AE150 automatic enzyme immunoassay system (Aikang Biotechnology Co. Ltd., Shenzhen, China). The ELISA kits of anti-TP testing were anti-TP diagnostic kits from Beijing Wantai (Wantai BioPharm, Beijing, China, sensitivity = 99.15%, specificity = 100%), Shanghai Kehua (Kehua Bioengineering Co. Ltd, Shanghai, China, sensitivity = 98.31%, specificity = 100%), and Xiamen Xinchuang (InTec PRODUCTS INC, Xiamen, China, sensitivity = 100%, specificity = 97.91%). The test kits were approved by the Chinese Food and Drug Administration. If both results of the two kits were negative, the sample was classified as negative. On the contrary, positive. If one result was negative and the other is reactive, then repeat the test in duplicate with the same kits and same equipment for the sample. If one or two of the repeated results were positive (repeat reactive), the sample was classified as positive.16 The testing algorithm of *Treponema pallidum*
antibody is shown in Figure 1. TPPA tests were performed on ELISA anti-TP positive cases since 2021 for confirmation. The kits of TPPA were from Zhuhai Livzon (SERODIA-TPPA, Livzon, Zhuhai, China), and the tests were performed manually. All the testing procedures strictly followed the standard operational procedures and the rules of Laboratory Quality Management and Regulation in Blood Stations of China.

Data Analysis
Socio-demographic data (sex, age, education and occupation), donation history and laboratory results were obtained from the database of blood management software of Jinan Blood Center. The data were sorted with Excel and analyzed with SPSS version 22 software. We calculated the overall and subgroup syphilis seropositivity ratio and its 95% CI. Binary logistic regression was used to determine the association factors with syphilis. ORs and their 95% CIs were calculated when comparing the risk of syphilis seropositivity. P < 0.05 was taken as statistically significant. χ² test was used to evaluate differences between males and females at different age stage and P<0.05 was considered as statistically significant.

Ethical Considerations
We declare that our study complies with the Declaration of Helsinki. Ethics approval was obtained from the Ethics Committee of Jinan Blood Center (No. 2021003). Written informed consent at the time of blood donation was obtained from all blood donors. All the study participants strictly observed the principle of confidentiality.

Results
Socio-Demographic Characteristics of the Blood Donors
Totally 700,757 blood donors were included in this study, and 72.0% (504,445) of them were male and 28.0% (196,312) were female. 37.39% of the donors aged 18–24 (261,982), next is 25–34 (182,462, 26.04%). As to education, 60.10% (421,123) of the donors received an education above high school. Of occupations reported, 15.23% (106,743) were students and 9.0% (63,053) were farmers. The majority (591,817, 84.45%) of the donors were first-time donors (Table 1).

Sero-Prevalence of Syphilis
All samples from 700,757 blood donors underwent laboratory test by ELISA. A total of 2290 blood donors were detected positive for anti-TP in our study, among which 1809 cases were positive in both tests (Wantai and Kehua or Wantai and Xinchuang), with a ratio of 79.0% (1809/2290). And 1924 cases were positive by one kit and were tested repeated in duplicate with the same kit. Among them, 481 cases were repeat reactive, with a repeat reactive ratio of 25.0% (481/
The confirmation test of TPPA showed that 139 out of 191 ELISA anti-TP positive cases were positive, with a co-positive rate of 72.77% (139/191). The seropositive rate of anti-TP among blood donors showed a two-phase change, with an upward trend in the years from 2007 to 2010 and a downward trend from 2014 to 2021, totaling 15 years. In 2007, the incidence was 0.32%. From 2008 to 2010, the incidence increased rapidly and reached 0.56% in 2010 and kept about 0.55% for 4 years. And after we conducted rapid screening of Treponema pallidum antibody before donation in 2014, the incidence began to decrease. The incidence decreased below 0.3% in 2016 and maintained a level of about 0.25% from then on. The overall positive rate of anti-TP was 326.8 per 100,000 (95% CI, 326.0 to 327.6). The prevalence of syphilis was 423.8 per 100,000 (95% CI, 403.55 to 444.1) among female blood donors compared to 289.0 per 100,000 (95% CI, 278.6 to 299.5) in male donors. Donors aged 35–44 years old showed a higher incidence (436.7 per 100,000, 95% CI, 413.8 to 459.7) than other groups. The study also showed those accepted a lower education below high school had a higher incidence of syphilis (415.5 per 100,000, 95% CI, 398.7 to 432.4). Among the known occupations, farmers (388.6 per 100,000, 95% CI, 354.2 to 422.9) and government employees (320.2 per 100,000, 95% CI, 283.4 to 357.0) had higher incidence than other occupations. Prevalence of first-time donors (361.9 per 100,000, 95% CI, 351.1 to 372.8) was higher than repeated donors (135.9 per 100,000, 95% CI, 120.4 to 151.3) (Table 2).

Logistic regression analysis showed that syphilis infection was associated with gender, age, education, first-time donor status and occupation. The high-risk factors were female, 35–44 years, high school education or lower, and occupation. Among the different kinds of occupations, farmers had the highest risk of being affected. Female donors were more likely to be positive for syphilis infection than male donors (COR = 1.468, 95% CI = 1.348–1.599, P < 0.001). Compared with 18–24 years old group, 25–34 years old group (COR = 1.623, 95% CI = 1.449–1.818), 35–44 years old group (COR = 2.026, 95% CI = 1.813–2.264) and 45–60 years old group (COR = 1.862, 95% CI = 1.636–2.118) had significantly (P < 0.001) higher seroprevalence than 18–24 years old group. Educational status was significantly associated with syphilis infection. Those

| Characteristics | N (%) |
|-----------------|-------|
| **Sex**         |       |
| Male            | 504,445 (72.0) |
| Female          | 196,312 (28.0) |
| **Age group**   |       |
| 18–24           | 261,982 (37.39) |
| 25–34           | 182,462 (26.04) |
| 35–44           | 158,673 (22.64) |
| 45–60           | 97,640 (13.93) |
| **Education**   |       |
| >High school    | 421,123 (60.10) |
| ≤High school    | 279,634 (39.90) |
| **Occupation**  |       |
| Students        | 106,743 (15.23) |
| Government employees | 45,282 (6.46) |
| Other occupations | 94,514 (13.49) |
| Farmers         | 63,053 (9.00) |
| Doctors         | 31,430 (4.49) |
| N.A.            | 359,735 (51.33) |
| **Donor history** |     |
| First time      | 591,817 (84.45) |
| Repeat and regular | 108,940 (15.55) |

Abbreviations: N.A. not available.
blood donors received less education (≤high school) (COR = 1.555, 95% CI = 1.433–1.688, P < 0.001) were at higher risk of syphilis infection compared to those who earned an education degree above high school. Among occupations reported, government employees (COR = 2.038, 95% CI = 1.631–2.546), workers (COR = 1.77, 95% CI = 1.454–2.143) and farmers (COR = 2.465, 95% CI = 2.025–3.0) had a significant higher risk of syphilis infection than students (P < 0.001). Donor history also showed significant difference between first-time donors (COR = 2.67, 95% CI = 2.26–3.155, P < 0.001) and repeated and regular donors.

We compared the syphilis seroprevalence between male and female donors. The seroprevalence of both groups increased with age. The high-risk age group of male donors was 35–44 years old subgroup, next is 45–60 years old subgroup. And the high-risk age group of female donors was 35–44 years old subgroup, next is 25–34 years old subgroup. Within every age subgroup, the prevalence of female donors was significantly higher than that of male donors (Table 3).

**Discussion**

In our study, confirmation test with TPPA showed that 72.77% ELISA positive cases were also TPPA positive. The results of ELISA and TPPA in this study had a relatively high coincidence, which indicates that the seropositive rate of anti-TP by ELISA test can largely reflect the incidence of syphilis infection among blood donation population in our city. Our study shows that the overall seroprevalence of *T. pallidum* seropositivity among blood donors from 2007 to 2021 in Jinan city was 326.8 per 100,000. Compared with data reported by other areas in China, the result was lower than most cities including economically developed areas in Southeast China such as Yangpu District of Shanghai (805 per
Foshan in Guangdong (380 per 100,000),\(^{18}\) Guangzhou in Guangdong (570 per 100,000),\(^{19}\) Maoming in Guangdong (550 per 100,000),\(^{20}\) Bao-an district of Shenzhen in Guangdong (520 per 100,000)\(^{21}\) and some cities in central and western China like Xian in Shanxi (910 per 100,000),\(^{22}\) Lanzhou in Gansu (590 per 100,000),\(^{23}\) and northeast cities like Changchun in Jilin (600 per 100,000)\(^{24}\) as well. The prevalence was similar to Taiyuan in Shanxi (300 per 100,000),\(^{25}\) Heze in Shandong (300 per 100,000),\(^{26}\) and Nanjing in Jiangsu (350 per 100,000).\(^{27}\) Variation of the total sample size, the study period, syphilis seroprevalence among the local general population and the test kits used may account for the discrepancy among various studies. The reason why the seroprevalence of syphilis in Jinan was lower than many other cities in China may mainly lie in two aspects: one was, as was reported, the prevalence of syphilis infection of Shandong was lower than the national average ratio;\(^{12}\) the other was, rapid testing of syphilis antibody before donation was taken since 2014 to battle against the dramatic rising trend of syphilis seroprevalence during 2007–2014. Compared to other countries’ report, our result was lower than Ethiopia (1820 per 100,000),\(^{28}\) and India (1623.7 per 100,000),\(^{29}\) but higher than Israel (47 per 100,000),\(^{30}\) USA (54.6 per 100,000)\(^{11}\) and Brazil (140 per 100,000).\(^{32}\)

Our study indicated that syphilis seroprevalence among female donors was higher than among male donors, and the incidence significantly increased with age. The seroprevalence of 35–44 years old group was higher than other age groups. And in every age subgroup, the seroprevalence of female donors was higher than that of male donors, the difference between them was significant (P < 0.05). The result was consistent with some previous studies.\(^{17,19,21,25}\) Why were women under bigger risk of being affected by *Treponema pallidum*? Unprotected sexual behavior made women more likely to be affected.\(^{33}\) Some previous studies found that females were more likely to be affected by STDs partly because of the different physiology and anatomy of the genital organs between both sexes.\(^{34}\) Studies have proved that the male-to-female transmission rate was higher than the female-to-male rate in certain STDs, such as HIV.\(^{34,35}\) A study among volunteer blood donors in Chengdu, China, reported that shared razors, ear piercings, and tattoos were also the high-risk factors for syphilis infection.\(^{36}\) They speculated that sharing cosmetic instruments may be a high-risk factor. The finding was novel, but there are also some caveats. As is known, *Treponema pallidum* is very sensitive to the environment, especially heat and dryness. Although blood-borne diseases may be transmitted by contaminated instruments during invasive procedures, this is proven for viruses but not so common for TP. It still remains unclear whether or not syphilis could survive on non-disinfected instruments, as well as how long it can survive. As cosmetic treatment becomes more and more popular, we should pay more attention to donors who may accept simple cosmetic operations like ear piercings, eyebrow tattoos and tattoos during recruitment, as such simple cosmetic operations may be performed in beauty salons or even on the street, where complete cleaning and disinfection of the instruments could hardly be executed.

Of the occupations reported, farmers had the highest seropositivity ratio. Many donors reported their occupations as farmers, but they might probably be migrant workers. With the rapid economic development of China, a large number of farmers left their land to work in towns or cities. They were called migrant workers. Migrant workers generally received less education, often below high school, and they had poor knowledge about the prevention of sexually transmitted diseases (STDs). Young adults of sexually active age, lack of STD knowledge, usually live separated from spouse, all these factors made migrant workers under greater risk of being affected by STDs than other population group.\(^{37}\) A surprising result of our study was that, unlike many other studies,\(^{11,12}\) government employees showed a high risk of

### Table 3 Male and Female Syphilis Seroprevalence in Different Age Groups at Jinan Blood Center, China, 2007–2021

| Variables | Male | | | Female | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | Number of Donors | Number of Seropositives | Prevalence % | Number of Donors | Number of Seropositives | Prevalence % | | | | | |
| 18–24 | 162,653 | 281 | 0.17 | 99,329 | 285 | 0.29 | 37,284 | 0.000 |
| 25–34 | 142,941 | 412 | 0.29 | 39,521 | 227 | 0.57 | 72,642 | 0.000 |
| 35–44 | 123,933 | 483 | 0.39 | 34,740 | 210 | 0.60 | 28,781 | 0.000 |
| 45–60 | 74,918 | 282 | 0.38 | 22,722 | 110 | 0.48 | 5,057 | 0.025 |
syphilis infection. In China, government employees are regarded as the middle class of the society with good income and high education and usually have a happy family. They are not the high-risk group in people’s mind. The reasons for these phenomena remain elusive and definitely deserve some research.

The study also showed that those with a lower education experience had a higher syphilis seroprevalence, which was consistent with many other studies.18–21,23,25 With the development of society and increasing mobility of the population, it is essential for the public health institutions to disseminate the knowledge of STDs to low education population.

Our study indicated that first-time donors were more likely to have T. pallidum seropositivity than repeat donors. First-time donors were thought to offer a better estimate of infectious risk than that of the general population given that, unlike repeat donors, they have not been tested previously and were less affected by selection bias.31 Although after consultation and preliminary screening, first-time donors also showed a higher incidence of syphilis. The existence of asymptomatic period of syphilis might account for it.

Limitation of the Study
Because of history and local economic restrictions, confirmation tests were not carried out during the study period, which affects the accuracy of syphilis seroprevalence.

Conclusion and Recommendations
In conclusion, compared to many other cities of China, the prevalence of T. pallidum seropositivity among Jinan blood donors remains low. Female, 35–44 years old, with a lower education, farmers and first-time donors were associated with higher T. pallidum seropositivity. More attention should be paid on distinguishing high-risk subgroups and the selection of donors from low-risk groups. Further research should be conducted among women and government employees on their high seroprevalence of syphilis, aiming to find the reasons for this and the potential intervention.

Abbreviations
WHO, World Health Organization; SARS, Severe Acute Respiratory Syndrome; COVID-19, Corona Virus Disease 2019; AIDS, Acquired Immune Deficiency Syndrome; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immuno-deficiency virus; CI, Confidence interval; COR, Crude Odds Ratios; STD, Sexually transmitted disease; TP, Treponema pallidum; TTIs, Transfusion-transmissible infections; TPPA, Treponema pallidum particle agglutination assay.

Data Sharing Statement
The data that analyzed in this study are available upon reasonable request from the corresponding author.

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Author Contributions
All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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The authors declare that they have no conflicts of interest in this work.
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