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Title page

Association between syphilis prevalence and age: an analysis of surveillance data among blood donors in southern China, 2014-2017

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ABSTRACT

Objective: This study investigated the associations between syphilis prevalence and age among blood donors, and described the distribution of serological titres among syphilis-infected donors, aiming at confirming the syphilis epidemic characteristics and promoting effective interventions for older adults.

Methods: Data were obtained from the Shenzhen Program for Syphilis Prevention and Control in 2014-2017. Blood samples were screened using the enzyme-linked immunosorbent assay (ELISA), and confirmed using the *Treponema pallidum* particle agglutination assay (TPPA) and toluidine red unheated serum test (TRUST).

Results: Among 394 792 blood donors, 733 were TPPA and TRUST positive, and 728 were only TPPA positive. The overall prevalence of syphilis infection was 370.1 per 100 000 [95% confidence interval (CI), 351.1-389.0 per 100 000]; the prevalence of active infection was 185.7 per 100 000 (95% CI, 172.2-199.1 per 100 000). People aged ≥45 years displayed a prevalence of 621.8 per 100 000 in syphilis infection and 280.5 per 100 000 in active infection, which were 3.8 times and 2.4 times higher than that for people aged <25 years. The prevalence of syphilis infection (χ^2_{trend} = 311.9, p_{trend} < 0.001) and active infection (χ^2_{trend} = 72.1, p_{trend} < 0.001) increased significantly with age. After stratification of gender and year of donation , the increasing trend of prevalence with age remained(p_{trend} < 0.05), except for the prevalence of active infection in males (χ^2_{trend} = 0.923, p_{trend} = 0.337) and females (χ^2_{trend} = 0.224, p_{trend} = 0.636) in 2014. About 16.3% of patients aged ≥45 years had a TRUST titre of ≥1□8, lower than that of patients aged <25 years (51.3%) and 25-34 years (34.1%).

Conclusions: The findings confirm the high prevalence of syphilis among older adults, and suggest the need to increase awareness among healthcare providers and deliver more-targeted prevention interventions for older adults to promote early testing.

Strengths and limitations of this study

- This study described the syphilis prevalence among nearly 400 000 blood donors, including syphilis infection, active infection, and distribution of serological titres.
- Using trend analysis after stratification of gender and year of donation to examine the association between syphilis prevalence and age, the results confirmed the increasing trend of prevalence with age.
- Lack of information on syphilis prevalence between first-time donors and repeat donors is a limitation to this study.

MAIN TEXT

INTRODUCTION

The global population is ageing as a combined result of the demographic transition from high to low levels of fertility and mortality.[1] Population ageing increases the total global disease burden, with approximately 23% attributable to disorders in people aged ≥60 years.[2] Chronic non-communicable diseases, including cardiovascular disease, malignant neoplasms, and chronic respiratory diseases, are the leading contributors to disease burden in older people.[2] However, infectious diseases also considerably affect older people, as an increasing incidence of infectious diseases, such as human immunodeficiency virus (HIV) and syphilis, was shown from recent surveillance data.[3-5] This large disease burden in older people calls for improvements in the healthcare system and more investments and programs focusing on healthy ageing.[2]

Syphilis, caused by *Treponema pallidum*, is a chronic infection with diverse clinical manifestations occurring in distinct stages, and may lead to blindness, dementia, delirium, death, etc., if not treated immediately or adequately.[6] Syphilis can also aid the passage for HIV to invade, reduce the CD4 T-cell levels, and increase the viral load, thereby aggravating the harm of HIV.[7] Even though syphilis can be effectively treated by penicillin, about 36.4 million new cases occur annually.[8] In China, the syphilis epidemic has rapidly increased, with 16.3% increase per year during the first decade after the SARS outbreak.[9] The reported incidence was slightly higher among females than males (ratio, 1.00 to 0.92), but it varied significantly with age.[5] The younger people (20-39 years) reported the highest syphilis incidence and accounted for the largest proportion of newly reported cases; however, the older age groups (≥45 years) had the fastest growth in incidence, and males aged ≥60 years displayed a peak incidence of latent syphilis in the last decade.[5] With the accelerated ageing of the global population, the increasing syphilis epidemic among older adults is alarming.

Shenzhen, located in southern China and next to Hong Kong, is a large city with a population of >10 million.[10] It was the first city in China to make donated blood meet

the demand for clinical use.[11] After China initiated the 10-year plan for syphilis prevention and control, Shenzhen launched a comprehensive program, the Shenzhen Program for Syphilis Prevention and Control (SPSPC), in November 2013 to enhance syphilis screening among blood donors and other five subgroups [HIV voluntary counsellors, methadone maintenance treatment users, female sex workers, men who have sex with men(MSM), and women of childbearing age], as well as case management, including diagnosis, treatment, and follow-up, for syphilis-infected adults.[12] The reported syphilis incidence remained relatively stable since 2008. However, consistent with the aforementioned characteristics of varied age groups, a rapid growth of syphilis incidence was observed among older adults in Shenzhen.[13] Studies usually considered blood donors as the representative of general population and used the prevalence data of blood donors for real-time surveillance and identification of high-risk groups.[14] Whether the syphilis seroprevalence among blood donors agrees with reported incidence characteristics remains to be studied. Therefore, this study aimed to examine differences in syphilis prevalence among blood donors and describe the distribution of serological titres among syphilis-infected donors with respect to age groups, to confirm the syphilis epidemic characteristics in southern China and support the design of effective interventions for older adults.

METHODS

Subjects and blood donation process

Blood donors were recruited by the Shenzhen Blood Center between 2014 and 2017. More than 10 blood mobiles, with the Shenzhen Blood Center logo and the words 'non-remunerated blood donation', were dispatched around the city to increase the accessibility of blood donation. Volunteers could go to the mobiles or to the blood centre directly.

Before donation, all potential donors needed to complete a detailed health history questionnaire, sign a donation registration form, undergo weight, blood pressure and heart rate measurement, and have a rapid test for blood type, hemoglobin, hepatitis B surface antigen and alanine transaminase. People who conformed to the Whole

Blood and Component Donor Selection Requirements (GB 18467-2001) could proceed to donate blood. All blood donors were non-remunerated. A light refreshment, a blood donation certification, and a blood credit allowing free transfusion for donors or their direct relatives were provided as incentives. The donation process and blood management were fully in accordance with the Blood Donation Law of the People's Republic of China and Blood Donation Regulation of the Shenzhen Special Economic Zone.

Serological testing

After donation, the blood samples were transferred to the Shenzhen Blood Center and underwent a series of laboratory testing. The enzyme-linked immunosorbent assay (ELISA; Zhuhai Lizhu Bio-engineering Co., Ltd., Zhuhai, China) was performed on all blood samples for syphilis screening. Syphilis-positive samples, with a form listing the donors' name, age, and gender, were then transferred to the Shenzhen Center for Chronic Disease Control [SZCCC, a city-level prevention and control centre for sexually transmitted diseases (STDs)] under SPSPC guidelines. A treponemal test of *Treponema pallidum* particle agglutination (TPPA; Fujirebio Inc., Tokyo, Japan) and a non-treponemal test of toluidine red unheated serum test (TRUST; Shanghai Rongsheng BioTech Co., Ltd., Shanghai, China) were used in the SZCCC to confirm the infection status. TRUST-positive samples further underwent a quantitative titre testing to monitor response to treatment. TPPA and TRUST results were sent back to the Shenzhen Blood Center in 2 days after the samples were received.

Definition of syphilis infection

Based on serological testing results, syphilis infection was divided into two categories: historical infection and active infection. Historical infection was defined as TPPA positive but TRUST negative, and active infection as both TPPA and TRUST positive.[15] The overall syphilis infection was defined as TPPA positive, including both historical and active infection. Moreover, high-titre was defined as active infection patients with a quantitative titre of ≥ 1∶8.

Statistical analysis

Primary outcomes of interest were prevalence of syphilis infection and active infection among all blood donors in different age groups. There were four age groups, <25 years, 25-34 years, 35-44 years, and ≥45 years, fully considering the age coverage of blood donors and age classification in previous studies.[16,17] We calculated the crude prevalence and its 95% confidence interval (CI). The chi-squared (χ^2) test for trend was used to assess the prevalence difference among age groups. Line graphs were used to describe the change in prevalence for both syphilis infection and active infection among the age groups after stratification of gender and year of donation. Odds ratios (ORs) and their 95% CIs were calculated when comparing the risk of syphilis infection and active infection between the ≥ 45 years age group and other age groups. Furthermore, we described the distribution of TRUST titres among age groups and compared the difference using the χ^2 test. Data were analysed using SPSS 17.0 for Windows; $p < 0.05$ was considered statistically significant in the χ^2 test.

RESULTS

Demographic characteristics

Between 2014 and 2017, a total of 394 792 donors were recruited by the Shenzhen Blood Center for non-remunerated blood donation. Among them, 67.4% were male and 85.0% were <45 years. The proportion of male donors increased significantly by age (χ^2_{trend} = 8301.1, $p_{trend} < 0.001$), and the number of donations in each age group increased over the studied years (χ^2_{trend} = 932.3, $p_{trend} < 0.001$) (**Table 1**).

Table 1 Characteristics of blood donors in different age groups in Shenzhen, 2014-2017

Variables	Aged <25 years (n=95736)	Aged 25-34 years (n=137447)	Aged 35-44 years (n=102422)	Aged ≥45 years (n=59187)	χ^2_{trend}	p_{trend} value
Gender					8301.1	<0.001
Male	51409(53.7%)	96237(70.0%)	74445(72.7%)	44061(74.4%)		
Female	44327(46.3%)	41210(30.0%)	27977(27.3%)	15126(25.6%)		
Year of donation					932.3	<0.001
2014	22389(23.4%)	31929(23.2%)	23131(22.6%)	11210(18.9%)		
2015	24330(25.4%)	33096(24.1%)	24011(23.4%)	13241(22.4%)		
2016	24560(25.7%)	35736(26.0%)	26362(25.7%)	15843(26.8%)		
2017	24457(25.5%)	36686(26.7%)	28918(28.2%)	18893(31.9%)		

Prevalence of syphilis infection and active infection

After ELISA testing, 2597 samples were positive and were sent to the SZCCC for further examination. Among them, 733 (28.2%) were both TPPA and TRUST positive, 728 (28.0%) were only TPPA positive, and 1136 (43.7%) were false positive (**Figure 1**). The overall prevalence of syphilis infection was 370.1 per 100 000 (95% CI, 351.1-389.0 per 100 000), and the prevalence of active infection was 185.7 per 100 000 (95% CI, 172.2-199.1 per 100 000). The prevalence of syphilis infection (478.1 vs 317.9 per 100 000, $\chi^2 = 60.4$, $p < 0.001$) and active infection (244.9 vs 157.1 per 100 000, $\chi^2 = 36.1$, $p < 0.001$) was higher among females than males and showed a decreasing trend from 2014 to 2017 (syphilis infection: $\chi^2_{trend} = 27.1$, $p_{trend} < 0.001$; active infection: $\chi^2_{trend} = 7.8$, $p_{trend} = 0.005$). People aged ≥45 years reported the highest prevalence of both syphilis infection and active infection, which was 3.8 times (OR = 3.8, 95% CI = 3.1-4.6) and 2.4 times (OR = 2.4, 95% CI = 1.9-3.0) higher than that among people aged <25 years, and 2.3 times (OR = 2.3, 95% CI = 2.0-2.6) and 1.8 times (OR = 1.8, 95% CI = 1.5-2.2) higher than that among people aged 25-34 years, respectively. Trend analysis shown that the prevalence of syphilis infection ($\chi^2_{trend} = 311.9$, $p_{trend} < 0.001$) and active infection ($\chi^2_{trend} = 72.1$, $p_{trend} < 0.001$) increased significantly with age (**Table 2**). After stratification of gender and year of donation, the increasing trend of prevalence with age remained ($p_{trend} < 0.05$), except for the prevalence of active infection in males ($\chi^2_{trend} = 0.923$, $p_{trend} = 0.337$) and females ($\chi^2_{trend} = 0.224$, $p_{trend} = 0.636$) in 2014 (**Figure 2**).

Table 2 Prevalence of syphilis infection and active infection among blood donors in different age groups

Age groups	Number of screened	Syphilis infection				Active infection			
		Number	Prevalence per 100 000 (95%CI)	χ^2_{trend}	p_{trend} value	Number	Prevalence per 100 000 (95%CI)	χ^2_{trend}	p_{trend} value
Aged <25 years	95736	158	165.0(139.3-190.8)	311.9	<0.001	113	118.0(96.3-139.8)	72.1	<0.001
Aged 25-34 years	137447	376	273.6(245.9-301.2)			211	153.5(132.8-174.2)		
Aged 35-44 years	102422	559	545.8(500.7-590.9)			243	237.3(207.5-267.0)		
Aged ≥45 years	59187	368	621.8(558.4-685.1)			166	280.5(237.9-323.1)		

Distribution of TRUST titres

Among 733 patients with active infection, a TRUST titre of 1:1 accounted for the largest proportion (41.7%), followed by a titre of 1:2 (24.1%). About 27.0% had a TRUST titre of ≥1:8. The distribution of TRUST titres was varied among the age groups (**Figure 3**). Patients aged ≥45 years comprised a large proportion of low titres at 1:1 and 1:2, and the proportion of high titres was only 16.3%, which was much smaller than that among patients aged <25 years (51.3%) and 25-34 years (34.1%) (**Table 3**).

Table 3 Proportion of high titres among active infection donors in different age groups

TRUST titres	Aged <25 years (n=113)	Aged 25-34 years (n=211)	Aged 35-44 years (n=243)	Aged ≥45 years (n=166)	χ^2	*p* value
< 1:8	55 (48.7%)	139 (65.9%)	202 (83.1%)	139 (83.7%)	61.7	<0.001
≥ 1:8	58 (51.3%)	72 (34.1%)	41 (16.9%)	27 (16.3%)		

DISCUSSION

This study identified that the overall prevalence of syphilis infection among nearly 400 000 blood donors in 2014-2017 was 370.1 per 100 000, which was higher than that reported in the United States (54.6 per 100 000) and Brazil (135.5 per 100 000),[14,18] but lower than that reported in Ethiopia (732.4 per 100 000), Cameroon (3976.3 per 100 000), and India (1623.7 per 100 000).[19-21] The prevalence was similar to that in many cities in mainland China, such as Xi'an (359.6 per 100 000), Urumqi (359.3 per 100 000), and Kunming (381.2 per 100 000).[16,22] However, unlike some studies using only one method (i.e., ELISA) to confirm the syphilis infection status and report the prevalence,[16,20,22,23] this study used ELISA as a screening test and then used TPPA and TRUST to confirm the serostatus if screened positive. Surprisingly, only 56.3% of ELISA-positive patients were confirmed by TPPA. The testing process in this study greatly increased the accuracy of syphilis prevalence. Furthermore, this study found that 50.2% (733/1461) of syphilis-infected donors were active infection patients, among which 27.0% (198/733) had a TRUST titre of ≥1:8. To our knowledge, this is the first in-depth study focusing on active infection and serological titre

distribution of syphilis among blood donors in mainland China. Here, the proportion of high titres among active infection patients was similar to that reported in the United States.[14]

This study found that syphilis prevalence significantly increased with age. Older adults aged ≥45 years displayed the highest prevalence of both syphilis infection and active infection. More importantly, from the national surveillance data, people aged ≥60 years had a remarkably higher increase in reported incidence compared with those aged 45-60 years.[5] Hospitalised patients aged ≥70 years showed the highest syphilis prevalence (4.8%), followed by patients aged 61-70 years (3.9%) and those aged 51-60 years (3.2%).[24] Given the results from this study and previous studies, health awareness and syphilis prevention focusing on older adults are needed.

The higher prevalence in older adults might be due to several reasons. First, many older people are sexually active,[25] and their sexual health and behaviour affect syphilis transmission. Low self-perception of risk and misconceptions or limited knowledge about syphilis and other STDs were frequently reported as reasons for condomless behaviours among older adults.[5,26] Second, older adults have been largely neglected by healthcare providers due to age-related stigma.[27] Sexual health services for HIV or STDs rarely focus on older adults, leaving this group behind in both testing and prevention. Third, presenting with a late diagnosis has been significantly associated with older age. Older people were more likely to be aware of their serostatus when in hospital or an active offer of a testing.[28] In this study, analysis of the TRUST titre distribution suggests that > 80% of people aged ≥45 years with low TRUST titres had a previous infection. However, late presentation is particularly worrying among older people because it further increases the risk of cardiovascular syphilis, neurosyphilis, paresis, etc. As syphilis is a great imitator, doctors often ignore syphilis infection when diagnosing the elderly, leading to omission of syphilis testing and misdiagnosis of the disease.

Evidence suggests that the most significant factor affecting testing patterns in older adults is providing the screening test actively.[28] Since the initiation of China's national

syphilis control plan, syphilis screening has been widely integrated into HIV voluntary counselling and testing (VCT) services. More than 95% of people who received HIV testing services have undergone free syphilis testing.[29] Referral, treatment, and follow-up services would be provided to those diagnosed with syphilis. In Shenzhen, more than half of VCT sites are set in community health service centres, where a separate room is arranged for counselling and testing service. However, due to the low awareness of self-testing, older adults rarely positively seek the services. Meanwhile, most health staff are unwilling to provide the service actively because of limited experience, lack of time, discomfort in discussing sexual behaviours and STDs with older adults, stigma, ageism, etc.[27] Hence, enhanced training of healthcare providers and education of older adults are necessary.

As mentioned, the prevalence of both syphilis infection and active infection were higher among females than males, consistent with the results of some previous studies.[22,23] The physiology and anatomy of the genital organs are much different between both sexes. Females are more likely to contract STDs in receptive vaginal sex behaviours.[30] The male-to-female transmission rate is higher than the female-to-male rate in certain STDs, such as HIV.[30,31] Besides, a proportion of females have premarital or extramarital sexual partners. A previous study has found that syphilis prevalence among husbands of 2261 syphilis-infected pregnant women was < 30%.[32] Additionally, being serofast (or remaining positive in a non-treponemal test and having a titre with less than a fourfold decline in ≥1 year after recommended therapy) is common among syphilis patients.[33] The serofast rate is higher among females than males (42.8% vs 28.6%),[34] leading to more females staying in the state of active infection. The exact mechanism of this difference is unclear, but it may be partly associated with the varied immune system between both sexes.[34] MSM are considered to be a major high-risk subgroup of syphilis infection and are permanent deferral of blood donation in China.[35] In this study, males were excluded if they reported they had ever had homosexual behaviours or had multiple sex partners in health history questionnaire, which may be one of the reasons for the low syphilis prevalence in males.

Limitations

Our study has several limitations. First, limited financial and human resources restricted us in using a population-based design, which is considered as the gold standard in evaluating disease epidemics.[14] The choice of blood donors as population samples may result in potential bias, such as selection bias for age coverage and self-identified health conditions. Second, this study did not distinguish the syphilis prevalence between first-time donors and repeat donors, which may lead to underestimation of syphilis disease burden. In previous studies, the syphilis prevalence among first-time donors was significantly higher than that among repeat donors.[14,16] Third, false-negative results attributable to the window period of syphilis infection may result in an underestimation of syphilis prevalence. However, the residual risk of syphilis infection is very low according to a residual risk analysis in Shenzhen.[36]

Conclusions

This study provides an in-depth analysis of the associations between syphilis prevalence and age. Older adults showed a high prevalence of both syphilis infection and active infection but a small proportion of high titres, which point towards the compelling need to heighten awareness among healthcare providers and deliver more-targeted prevention interventions for older adults to promote early testing.

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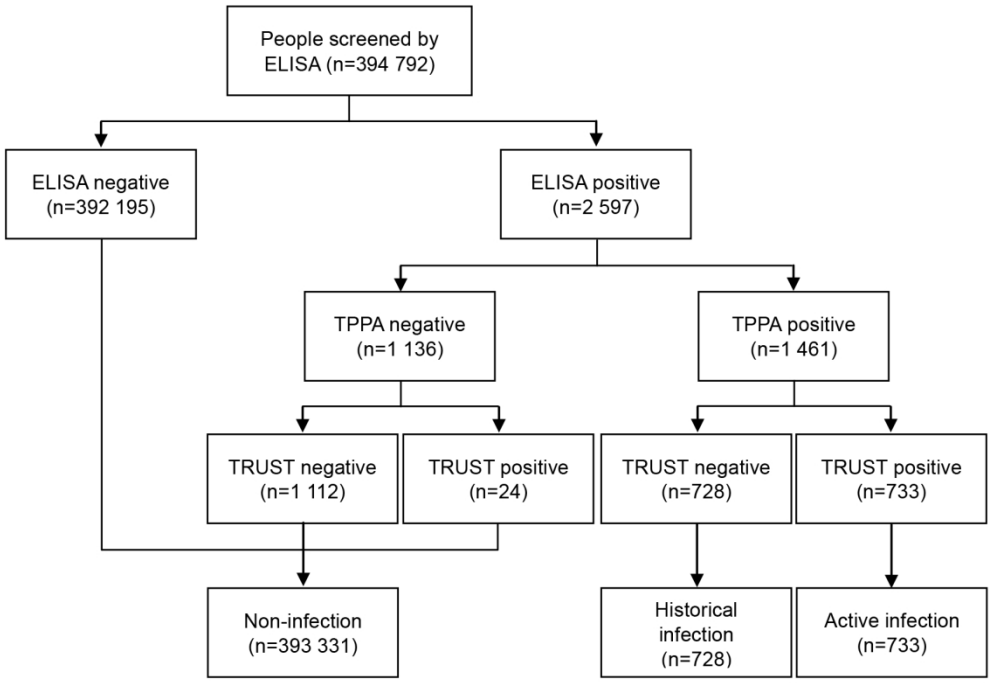

Figure 1 Flowchart for syphilis screening and confirmatory testing among blood donors. ELISA, enzyme-linked immunosorbent assay; TPPA, Treponema pallidum particle agglutination assay; TRUST, toluidine red unheated serum test.

157x116mm (300 x 300 DPI)

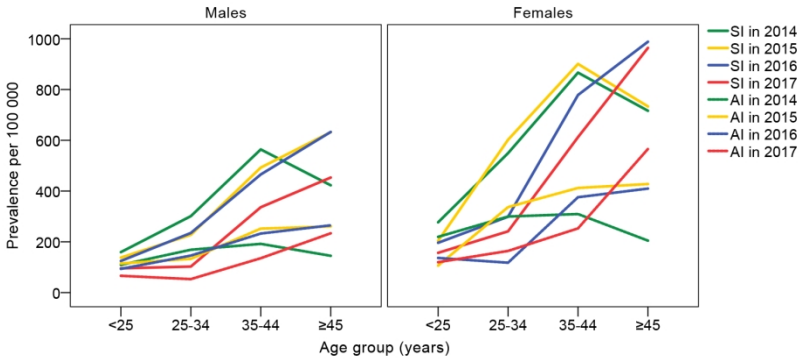

Figure 2 Prevalence of syphilis infection and active infection in different age groups, 2014-2017. (A) Prevalence of syphilis infection and active infection among males. (B) Prevalence of syphilis infection and active infection among females. SI: syphilis infection; AI: active infection

166x78mm (300 x 300 DPI)

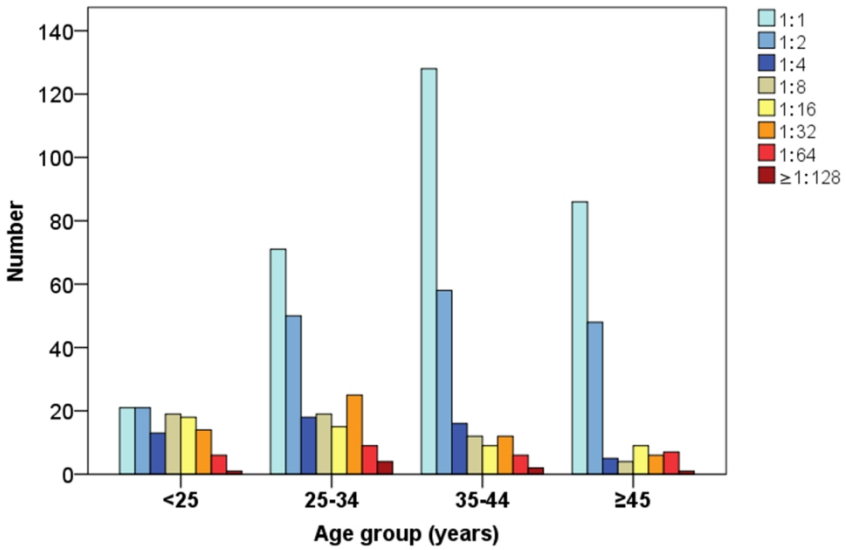

Figure 3 Distribution of TRUST titres among active infection donors in different age groups.

159x91mm (300 x 300 DPI)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	P1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	P5
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	P7-8
Objectives	3	State specific objectives, including any prespecified hypotheses	P8
Methods			
Study design	4	Present key elements of study design early in the paper	P8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	P8-9
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	P8-9
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	P9
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	P9
Bias	9	Describe any efforts to address potential sources of bias	*P9*
Study size	10	Explain how the study size was arrived at	Not applicable
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	P10
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	P10
		(*b*) Describe any methods used to examine subgroups and interactions	P10
		(*c*) Explain how missing data were addressed	Not applicable
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	Not applicable
		(*e*) Describe any sensitivity analyses	Not applicable
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	P11
		(b) Give reasons for non-participation at each stage	Not applicable
		(c) Consider use of a flow diagram	P20
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	P10
		(b) Indicate number of participants with missing data for each variable of interest	Not applicable
Outcome data	15*	Report numbers of outcome events or summary measures	P11,21
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	P11-12, 21,22
		(*b*) Report category boundaries when continuous variables were categorized	P10
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	P11
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Not applicable
Discussion			
Key results	18	Summarise key results with reference to study objectives	P12-14
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	P15
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	P15
Generalisability	21	Discuss the generalisability (external validity) of the study results	P15
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	P16

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Title page

Association between syphilis seroprevalence and age among blood donors in southern China: an observational study from 2014 to 2017

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ABSTRACT

Objective: This study investigated the association between syphilis seroprevalence and age among blood donors, and described the distribution of serological titres among syphilis-infected donors, aiming to confirm the syphilis epidemic characteristics and to promote effective interventions for older adults.

Methods: Data were obtained from the Shenzhen Program for Syphilis Prevention and Control in 2014-2017. Blood samples were screened using the enzyme-linked immunosorbent assay (ELISA), and confirmed using the *Treponema pallidum* particle agglutination assay (TPPA) and toluidine red unheated serum test (TRUST).

Results: Among 394 792 blood donors, 733 tested TPPA and TRUST positive (active infection), and 728 tested only TPPA positive (historical infection). The overall prevalence of syphilis infection was 370.1 per 100 000 [95% confidence interval (CI), 351.1-389.0 per 100 000]; the prevalence of active infection was 185.7 per 100 000 (95% CI, 172.2-199.1 per 100 000). People aged ≥45 years displayed a prevalence of 621.8 per 100 000 in syphilis infection and 280.5 per 100 000 in active infection, which were 3.8 times and 2.4 times higher than that for people aged <25 years, respectively. The prevalence of syphilis infection (χ^2_{trend} = 311.9, p_{trend} < 0.001) and active infection (χ^2_{trend} = 72.1, p_{trend} < 0.001) increased significantly with age. After stratification by gender and year of donation , the increasing trend of prevalence with age remained(p_{trend} < 0.05), except for the prevalence of active infection in males and females in 2014. About 16.3% of donors with active infection and aged ≥45 years had a TRUST titre of ≥1:8, lower than that of patients aged <25 years (51.3%) and 25-34 years (34.1%).

Conclusions: The findings confirm the high prevalence of syphilis among older adults, and suggest the need to increase awareness among healthcare providers and deliver more targeted prevention interventions for older adults to promote early testing.

Strengths and limitations of this study

- This study described the syphilis seroprevalence among nearly 400 000 blood donors, including syphilis infection, active infection, and distribution of serological titres.
- The testing process in this study, using enzyme-linked immunosorbent assay (ELISA) as a screening test and then using *Treponema pallidum* particle agglutination assay (TPPA) and toluidine red unheated serum test (TRUST) to confirm the serostatus, increased the accuracy of syphilis seroprevalence.
- Lack of information on syphilis prevalence between first-time donors and repeat donors was a limitation to this study.

MAIN TEXT

INTRODUCTION

The global population is ageing as a combined result of the demographic transition from high to low levels of fertility and mortality.[1] Population ageing increases the total global disease burden, with approximately 23% attributable to disorders in people aged ≥60 years.[2] Chronic non-communicable diseases, including cardiovascular disease, malignant neoplasms, and chronic respiratory diseases, are the leading contributors to disease burden in older people.[2] However, infectious diseases also considerably affect older people, as an increasing incidence of infectious diseases, such as human immunodeficiency virus (HIV) and syphilis, was shown from recent surveillance data.[3-5] This large disease burden among older people calls for improvements in the healthcare system and more investments and programs focusing on healthy ageing.[2]

Syphilis, caused by *Treponema pallidum*, is a chronic infection with diverse clinical manifestations occurring in distinct stages, and may lead to blindness, dementia, delirium, death, etc., if not treated immediately or adequately.[6] Syphilis can also aid the passage for HIV to invade, reduce the CD4 T-cell levels, and increase the viral load, thereby aggravating the harm caused by HIV.[7] Even though syphilis can be effectively treated with penicillin, about 36.4 million new cases occur annually.[8] In China, the syphilis epidemic has rapidly increased, with 16.3% increase per year during the first decade after the severe acute respiratory syndrome (SARS) outbreak.[9] The reported incidence was slightly higher among females than males (ratio, 1.00 to 0.92), but it varied significantly with age.[5] Younger people (aged 20-39 years) reported the highest syphilis incidence and accounted for the largest proportion of newly reported cases; however, the older age groups (aged ≥45 years) had the fastest growth in incidence, and males aged ≥60 years displayed a peak incidence of latent syphilis in the last decade.[5] With the accelerated ageing of the global population, the increasing syphilis epidemic among older adults is alarming.

Shenzhen, a special economic zone located in southern China and with a population of >10 million, is one of the cities that most affected by syphilis. The reported incidence of syphilis was over 60 per 100 000 in last 10 years, which was much higher than the national incidence. [5,10] Consistent with the aforementioned characteristics of varied age groups, a rapid increase in syphilis incidence was observed among older adults in Shenzhen.[11] Studies usually considered blood donors as a representative of the general population and used the prevalence data of blood donors for real-time surveillance and identification of high-risk groups.[12] Whether the syphilis seroprevalence among blood donors agrees with reported incidence characteristics remains to be studied. Shenzhen launched a comprehensive program, the Shenzhen Program for Syphilis Prevention and Control (SPSPC), in November 2013 to enhance syphilis screening among blood donors and five other subgroups [HIV voluntary counsellors, methadone maintenance treatment users, female sex workers, men who have sex with men(MSM), and women of childbearing age], as well as case management, including diagnosis, treatment, and follow-up, for syphilis-infected adults.[13] Based on the data from the SPSPC, this study aimed to examine differences in syphilis seroprevalence among blood donors and describe the distribution of serological titres among syphilis-infected donors with respect to age groups, to confirm the syphilis epidemic characteristics in southern China and support the design of effective interventions for older adults.

METHODS

Subjects and blood donation process

Blood donors were recruited by the Shenzhen Blood Center between 2014 and 2017. About 10 blood mobiles, with the Shenzhen Blood Center logo and the words 'non-remunerated blood donation', were dispatched around the city to increase the accessibility of blood donation. Volunteers could either go to the mobiles or to the blood centre directly.

Before donation, all potential donors needed to complete a health history questionnaire, sign a donation registration form, and undergo a rapid testing and a brief physical examination. The health history questionnaire contained a total of 27 medical conditions that would permanently or temporarily prevent the donors from donation, including a series of chronic diseases and infectious diseases (e.g., HIV, syphilis), transplant, high-risk behaviours (e.g., homosexual behaviours, drug use), surgery, delivery, breastfeeding, etc. Pre-donation repaid testing included blood type, haemoglobin, hepatitis B surface antigen, and alanine transaminase. Weight, blood pressure, heart rate and body temperature were measured and clinical examination of the skin and limbs was conducted. People who conformed to the Whole Blood and Component Donor Selection Requirements (GB 18467-2001) could proceed to donate blood. All blood donors were non-remunerated. A light refreshment, a blood donation certification, and a blood credit allowing free transfusion for donors or their direct relatives were provided as incentives. The donation process and blood management were fully in accordance with the Blood Donation Law of the People's Republic of China and the Blood Donation Regulation of the Shenzhen Special Economic Zone.

Serological testing

After donation, the blood samples were transferred to the Shenzhen Blood Center and underwent a series of laboratory testing. The enzyme-linked immunosorbent assay (ELISA; Zhuhai Lizhu Bio-engineering Co., Ltd., Zhuhai, China) was performed on all blood samples for syphilis screening. Syphilis-positive samples, with a form listing the donors' name, age, and gender, were then transferred to the Shenzhen Center for Chronic Disease Control [SZCCC, a city-level control and prevention centre for sexually transmitted diseases (STDs)] under SPSPC guidelines. A treponemal test of *Treponema pallidum* particle agglutination (TPPA; Fujirebio Inc., Tokyo, Japan) and a non-treponemal test of toluidine red unheated serum test (TRUST; Shanghai Rongsheng BioTech Co., Ltd., Shanghai, China) were used at the SZCCC to confirm the infection status. TRUST-positive samples further underwent a quantitative titre testing to monitor

response to treatment. TPPA and TRUST results were sent back to the Shenzhen Blood Center within 2 days after the samples were received.

Definition of syphilis infection

Based on serological testing results, syphilis infection was divided into two categories: historical infection and active infection. Historical infection was defined as TPPA positive but TRUST negative and active infection as both TPPA and TRUST positive.[14] Overall syphilis infection was defined as TPPA positive, including both historical and active infection. Moreover, high-titre was defined as a quantitative titre of ≥ 1:8 in patients with active infection.

Statistical analysis

Primary outcomes of interest were the prevalence of syphilis infection and active infection among all blood donors in different age groups. There were four age groups, <25 years, 25-34 years, 35-44 years, and ≥45 years, fully considering the age coverage of blood donors and age classification in previous studies.[15,16] We calculated the crude prevalence and its 95% confidence interval (CI). The chi-squared (χ^2) test for trend was used to assess the prevalence difference among age groups. Odds ratios (ORs) and their 95% CIs were calculated when comparing the risk of syphilis infection and active infection between the ≥ 45 years age group and other age groups. Line graphs were used to describe the changes in prevalence for both syphilis infection and active infection among the age groups after stratification by gender and year of donation. Furthermore, we described the distribution of TRUST titres among the age groups and compared the difference using the χ^2 test for trend. Data were analysed using SPSS 17.0 for Windows; $p < 0.05$ was considered statistically significant in the χ^2 test.

Patient and public involvement statement

Patients and the public were not involved in developing the hypothesis or the research questions, nor were they involved in developing plans for the design or implementation

of this study. The staff of the Shenzhen Blood Center were responsible for telling syphilis-positive participants about the test results and providing referral services related to syphilis treatment and management by phone.

RESULTS

Demographic characteristics

From 2014 to 2017, a total of 394 792 donors were recruited by the Shenzhen Blood Center for non-remunerated blood donation. Among them, 67.4% were male and 85.0% were aged <45 years. The distribution of age was varied between genders (χ^2 = 11249.0, p < 0.001) and among years of donation (χ^2 = 1182.0, p < 0.001). People aged 25-34 years accounted for the largest proportion of donors (**Table 1**).

Table 1 Characteristics of blood donors in different age groups in Shenzhen, 2014-2017

Variables	Aged <25 years (n=95736)	Aged 25-34 years (n=137447)	Aged 35-44 years (n=102422)	Aged ≥45 years (n=59187)	χ^2	*p* value
Gender					11249.0	<0.001
Male	51409(19.3%)	96237(36.2%)	74445(28.0%)	44061(16.6%)		
Female	44327(34.5%)	41210(32.0%)	27977(21.7%)	15126(11.8%)		
Year of donation					1182.0	<0.001
2014	22389(25.3%)	31929(36.0%)	23131(26.1%)	11210(12.6%)		
2015	24330(25.7%)	33096(35.0%)	24011(25.4%)	13241(14.0%)		
2016	24560(24.0%)	35736(34.9%)	26362(25.7%)	15843(15.5%)		
2017	24457(22.4%)	36686(33.7%)	28918(26.5%)	18893(17.3%)		

Prevalence of syphilis infection and active infection

After ELISA testing, 2597 samples tested positive and were sent to the SZCCC for further examination. Among them, 733 (28.2%) were both TPPA and TRUST positive, 728 (28.0%) were only TPPA positive, and 1136 (43.7%) were false positive (**Figure 1**). The overall prevalence of syphilis infection was 370.1 per 100 000 (95% CI, 351.1-389.0 per 100 000), and the prevalence of active infection was 185.7 per 100 000 (95% CI, 172.2-199.1 per 100 000). The prevalence of syphilis infection and active infection was higher among

females than males (syphilis infection: χ^2 = 60.4, p < 0.001; active infection: χ^2 = 36.1, p < 0.001) and showed a decreasing trend from 2014 to 2017 (syphilis infection: χ^2_{trend} = 27.1, p_{trend} < 0.001; active infection: χ^2_{trend} = 7.8, p_{trend} = 0.005). People aged ≥45 years reported the highest prevalence of both syphilis infection and active infection, which was 3.8 times (OR = 3.8, 95% CI = 3.1-4.6) and 2.4 times (OR = 2.4, 95% CI = 1.9-3.0) higher than that among people aged <25 years, and 2.3 times (OR = 2.3, 95% CI = 2.0-2.6) and 1.8 times (OR = 1.8, 95% CI = 1.5-2.2) higher than that among people aged 25-34 years, respectively. Trend analysis showed that the prevalence of syphilis infection (χ^2_{trend} = 311.9, p_{trend} < 0.001) and active infection (χ^2_{trend} = 72.1, p_{trend} < 0.001) increased significantly with age (**Table 2**). After stratification by gender and year of donation, the increasing trend of prevalence with age remained (p_{trend} < 0.05), except for the prevalence of active infection among males (χ^2_{trend} = 0.923, p_{trend} = 0.337) and females (χ^2_{trend} = 0.224, p_{trend} = 0.636) in 2014 (**Figure 2**).

Table 2 Prevalence of syphilis infection and active infection among blood donors in different age groups

Age groups	Number of screened	Syphilis infection				Active infection			
		Number	Prevalence per 100 000 (95%CI)	χ^2_{trend}	p_{trend} value	Number	Prevalence per 100 000 (95%CI)	χ^2_{trend}	p_{trend} value
Aged <25 years	95736	158	165.0(139.3-190.8)	311.9	<0.001	113	118.0(96.3-139.8)	72.1	<0.001
Aged 25-34 years	137447	376	273.6(245.9-301.2)			211	153.5(132.8-174.2)		
Aged 35-44 years	102422	559	545.8(500.7-590.9)			243	237.3(207.5-267.0)		
Aged ≥45 years	59187	368	621.8(558.4-685.1)			166	280.5(237.9-323.1)		

Distribution of TRUST titres

Among 733 donors with active infection, a TRUST titre of 1∶1 accounted for the largest proportion (41.7%), followed by a titre of 1∶2 (24.1%). About 27.0% had a TRUST titre of ≥1∶8. The distribution of TRUST titres was varied among the age groups (**Figure 3**). Patients aged ≥45 years comprised a large proportion of low titres at 1∶1 and 1∶2, and the proportion of high titres was only 16.3%, which was much smaller than that among patients aged <25 years (51.3%) and 25-34 years (34.1%). The proportion of high-titre

declined significantly with age (χ^2_{trend} = 53.6, p_{trend} < 0.001) (**Table 3**).

Table 3 Proportion of high-titre among active infection donors in different age groups

Age groups	TRUST titre < 1∶8	TRUST titre ≥ 1∶8	χ^2_{trend}	p_{trend} value
Aged <25 years	55 (48.7%)	58 (51.3%)	53.6	<0.001
Aged 25-34 years	139 (65.9%)	72 (34.1%)		
Aged 35-44 years	202 (83.1%)	41 (16.9%)		
Aged ≥45 years	139 (83.7%)	27 (16.3%)		

DISCUSSION

This study identified that the overall seroprevalence of syphilis infection among nearly 400 000 blood donors in 2014-2017 was 370.1 per 100 000, which was higher than that reported in the United States (54.6 per 100 000) and Brazil (135.5 per 100 000),[12,17] but lower than that reported in Ethiopia (732.4 per 100 000), Cameroon (3976.3 per 100 000), and India (1623.7 per 100 000).[18-20] The prevalence was similar to that in many cities in mainland China, such as Xi'an (359.6 per 100 000), Urumqi (359.3 per 100 000), and Kunming (381.2 per 100 000).[15,21] However, unlike some studies that used only one method (i.e., ELISA) to confirm the syphilis infection status and report the prevalence,[15,19,21,22] this study used ELISA as a screening test and then used TPPA and TRUST to confirm the serostatus if screened positive. As is known, ELISA is a method used worldwide for syphilis screening, with a sensitivity of >98% and specificity of >99% according to the reagent evaluation.[23] TPPA is considered as the gold standard test in syphilis diagnosis. Surprisingly, only 56.3% of ELISA-positive patients in this study were confirmed by TPPA, meaning the positive predictive value (the value associated with sensitivity, specificity and disease prevalence) for ELISA was below 60%. The testing process in this study greatly reduced the number of false-positives and increased the accuracy of syphilis seroprevalence.

To our knowledge, this study is the first in-depth study focusing on active infection and

serological titre distribution of syphilis among blood donors in mainland China. Active infection is different from historical infection as the former indicates more transmission and late syphilis if without timely and adequate treatment. The higher the serological titre, the more the risk of transmission (e.g., mother-to-child transmission) and adverse outcomes.[24] This study documented that 50.2% (733/1461) of syphilis-infected donors had active infection patients, and 13.6% (198/1461) had a TRUST titre of ≥1:8. Here, the proportion of high titres among syphilis infection patients was similar to that reported in the United States.[12]

This study found that syphilis prevalence significantly increased with age. Older adults aged ≥45 years displayed the highest prevalence of both syphilis infection and active infection. More importantly, from the national surveillance data, people aged ≥60 years had a remarkably higher increase in reported incidence compared with those aged 45-60 years.[5] Hospitalised patients aged ≥70 years showed the highest syphilis prevalence (4.8%), followed by patients aged 61-70 years (3.9%) and those aged 51-60 years (3.2%), which was much different from that for HIV infection for which patients aged 31-40 years recorded the highest prevalence.[25] Based on the results of this study and previous studies, health awareness and syphilis prevention focusing on older adults are needed.

The higher prevalence in older adults might be due to several reasons. First, many older people are sexually active,[26] and their sexual health and behaviour affect syphilis transmission. Low self-perception of risk and misconceptions or limited knowledge about syphilis and other STDs were frequently reported as reasons for condomless sex among older adults.[5,27] Second, older adults have been largely neglected by healthcare providers due to age-related stigma.[28] Sexual health services for HIV or STDs rarely focus on older adults, leaving this group behind in both testing and prevention. Third, presenting with a late diagnosis has been significantly associated with older age. Older people were more likely to be aware of their serostatus when in hospital or had an

active offer for testing.[29] In this study, analysis of the TRUST results suggests that > 90% of syphilis-infected people aged ≥45 years with TRUST negative or with low titres had a previous infection. However, late presentation is particularly worrying among older people because it further increases the risk of cardiovascular syphilis, neurosyphilis, paresis, etc. As syphilis is a great imitator, doctors often ignore syphilis infection when diagnosing the elderly, leading to omission of syphilis testing and misdiagnosis of the disease.

Evidence suggests that the most significant factor affecting testing patterns in older adults is providing the screening test actively.[29] Since the initiation of China's national syphilis control plan, syphilis screening has been widely integrated into HIV voluntary counselling and testing (VCT) services. More than 95% of people who received HIV testing services have undergone free syphilis testing.[30] Referral, treatment, and follow-up services would be provided to those diagnosed with syphilis. In Shenzhen, more than half of VCT sites are set in community health service centres, where a separate room is arranged for counselling and testing service. However, due to the low awareness of self-testing, older adults rarely positively seek the services. Meanwhile, most health staff are unwilling to provide the service actively because of limited experience, lack of time, discomfort in discussing sexual behaviours and STDs with older adults, stigma, ageism, etc.[28] Hence, enhanced training of healthcare providers and education of older adults are necessary.

Consistent with the results of some previous studies, the prevalence of both syphilis infection and active infection were higher among females than males.[21,22] It may stem partly from the different physiology and anatomy of the genital organs between both sexes, leading to females being more likely to contract STDs in receptive vaginal sex behaviours.[31] Some studies have proved that the male-to-female transmission rate is higher than the female-to-male rate in certain STDs, such as HIV.[31,32] Besides, a proportion of females have premarital or extramarital sexual partners. A previous study

has found that the syphilis prevalence among husbands of 2261 syphilis-infected pregnant women was < 30%.[33] Additionally, being serofast [defined as remaining positive in a non-treponemal test and keeping the titre at a certain level (mostly 1:8 or below) after recommended therapy and follow-up at least 1 year for primary syphilis, 2 years for secondary syphilis and 3 years for late syphilis] is common among syphilis patients.[34] The serofast rate is higher among females than males (42.8% vs. 28.6%),[35] leading to more females staying in the state of active infection. The exact mechanism underlying this difference is unclear, but it may be partly associated with the varied immune system between both sexes.[35] Furthermore, MSM are considered to be a major high-risk subgroup for syphilis infection and are permanently deferred from blood donation in China.[36] In this study, males were excluded if they reported they had ever engaged in homosexual behaviour in the health history questionnaire, which may be one of the reasons for the low syphilis prevalence among males.

Limitations

Our study has several limitations. First, limited financial and human resources restricted us in using a population-based design, which is considered as the gold standard in evaluating disease epidemics.[12] The choice of blood donors as population samples may result in potential bias, such as selection bias for age coverage and self-identified health conditions. Second, this study did not distinguish the syphilis seroprevalence between first-time donors and repeat donors, which may lead to underestimation of syphilis disease burden. In previous studies, the syphilis seroprevalence among first-time donors was significantly higher than that among repeat donors.[12,15] Third, false-negative results attributable to the window period of syphilis infection may result in an underestimation of syphilis prevalence. However, the residual risk of syphilis infection is very low according to a residual risk analysis conducted in Shenzhen.[37]

Conclusions

This study provides an in-depth analysis of the association between syphilis

seroprevalence and age. Older adults showed a high prevalence of both syphilis infection and active infection but a small proportion of high titres, which point towards the compelling need to heighten awareness among healthcare providers and deliver more targeted prevention interventions for older adults to promote early testing.

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FIGURE LEGENDS

Figure 1 Flowchart for syphilis screening and confirmatory testing among blood donors. ELISA: enzyme-linked immunosorbent assay; TPPA: *Treponema pallidum* particle agglutination assay; TRUST: toluidine red unheated serum test.

Figure 2 Prevalence of syphilis infection and active infection in different age groups, 2014-2017. (A) Prevalence of syphilis infection and active infection among males. (B) Prevalence of syphilis infection and active infection among females. SI: syphilis infection; AI: active infection.

Figure 3 Distribution of TRUST titres among active infection donors in different age groups.

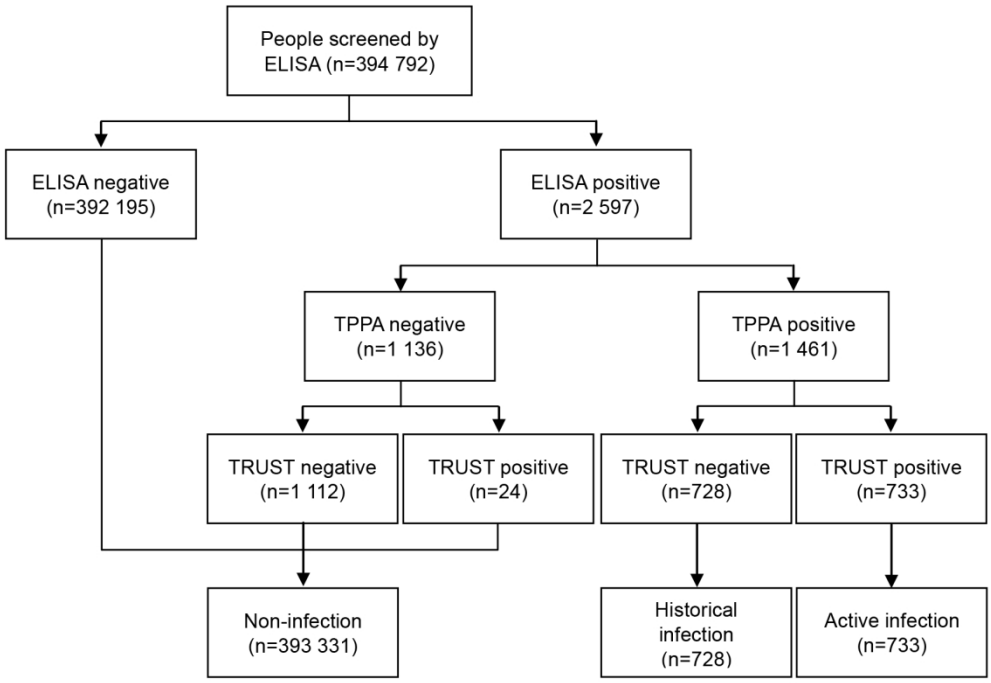

Figure 1 Flowchart for syphilis screening and confirmatory testing among blood donors. ELISA: enzyme-linked immunosorbent assay; TPPA: Treponema pallidum particle agglutination assay; TRUST: toluidine red unheated serum test.

157x116mm (300 x 300 DPI)

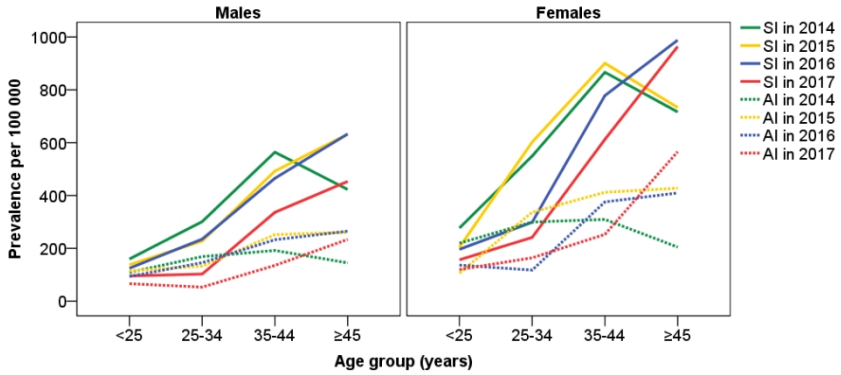

Figure 2 Prevalence of syphilis infection and active infection in different age groups, 2014-2017. (A) Prevalence of syphilis infection and active infection among males. (B) Prevalence of syphilis infection and active infection among females. SI: syphilis infection; AI: active infection.

423x182mm (72 x 72 DPI)

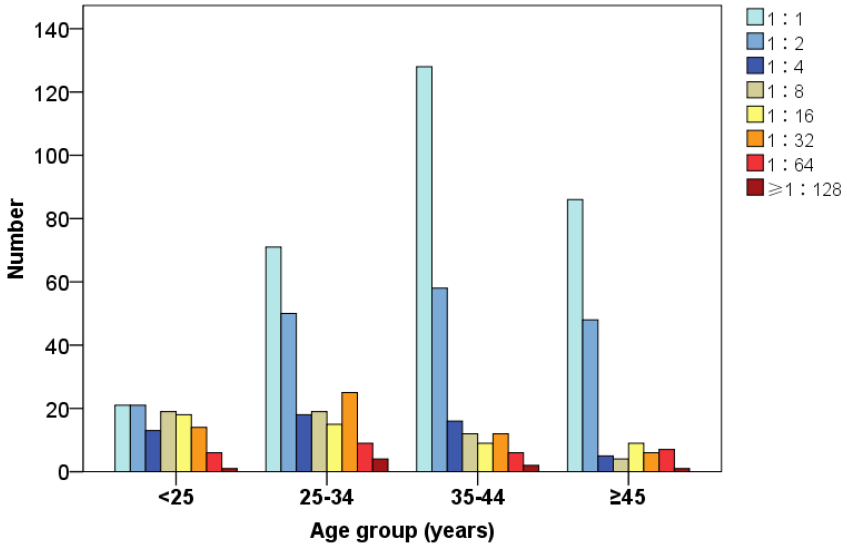

Figure 3 Distribution of TRUST titres among active infection donors in different age groups.

316x182mm (72 x 72 DPI)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	P1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	P6
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	P9-10
Objectives	3	State specific objectives, including any prespecified hypotheses	P10
Methods			
Study design	4	Present key elements of study design early in the paper	P10-11
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	P10-11
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	P10-11
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	P12
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	P11-12
Bias	9	Describe any efforts to address potential sources of bias	P11
Study size	10	Explain how the study size was arrived at	P10,P13
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	P12
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	P12
		(*b*) Describe any methods used to examine subgroups and interactions	P12
		(*c*) Explain how missing data were addressed	Not applicable
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	Not applicable
		(*e*) Describe any sensitivity analyses	Not applicable
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility,	P13

		confirmed eligible, included in the study, completing follow-up, and analysed	
		(b) Give reasons for non-participation at each stage	Not applicable
		(c) Consider use of a flow diagram	P24
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	P13
		(b) Indicate number of participants with missing data for each variable of interest	Not applicable
Outcome data	15*	Report numbers of outcome events or summary measures	P13-15
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	P13-15
		(*b*) Report category boundaries when continuous variables were categorized	P13
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	P14
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Not applicable
Discussion			
Key results	18	Summarise key results with reference to study objectives	P15
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	P18
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	P17-18
Generalisability	21	Discuss the generalisability (external validity) of the study results	P15-18
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	P19

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Association between syphilis seroprevalence and age among blood donors in southern China: an observational study from 2014 to 2017

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Title page

Association between syphilis seroprevalence and age among blood donors in southern China: an observational study from 2014 to 2017

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ABSTRACT

Objective: This study investigated the association between syphilis seroprevalence and age among blood donors, and described the distribution of serological titres among syphilis-infected donors, aiming to confirm the syphilis epidemic characteristics and to promote effective interventions for older adults.

Methods: Data were obtained from the Shenzhen Program for Syphilis Prevention and Control in 2014-2017. Blood samples were screened using the enzyme-linked immunosorbent assay (ELISA), and confirmed using the *Treponema pallidum* particle agglutination assay (TPPA) and toluidine red unheated serum test (TRUST).

Results: Among 394 792 blood donors, 733 tested TPPA and TRUST positive (active infection), and 728 tested only TPPA positive (historical infection). The overall prevalence of syphilis seropositivity was 370.1 per 100 000 [95% confidence interval (CI), 351.1-389.0 per 100 000]; the prevalence of active infection was 185.7 per 100 000 (95% CI, 172.2-199.1 per 100 000). People aged ≥45 years displayed a prevalence of 621.8 per 100 000 in syphilis seropositivity and 280.5 per 100 000 in active infection, which were 3.8 times and 2.4 times higher than that for people aged <25 years, respectively. The prevalence of syphilis seropositivity (χ^2_{trend} = 311.9, p_{trend} < 0.001) and active infection (χ^2_{trend} = 72.1, p_{trend} < 0.001) increased significantly with age. After stratification by gender and year of donation , the increasing trend of prevalence with age remained(p_{trend} < 0.05), except for the prevalence of active infection in males and females in 2014. About 16.3% of donors with active infection and aged ≥45 years had a TRUST titre of ≥1:8, lower than that of patients aged <25 years (51.3%) and 25-34 years (34.1%).

Conclusions: The findings confirm the high prevalence of syphilis among older adults, and suggest the need to increase awareness among healthcare providers and deliver more targeted prevention interventions for older adults to promote early testing.

Strengths and limitations of this study

- This study described the syphilis seroprevalence among nearly 400 000 blood donors, including syphilis seropositivity, active infection, and distribution of serological titres.
- The testing process in this study, using enzyme-linked immunosorbent assay (ELISA) as a screening test and then using *Treponema pallidum* particle agglutination assay (TPPA) and toluidine red unheated serum test (TRUST) to confirm the serostatus, increased the accuracy of syphilis seroprevalence.
- Lack of information on syphilis seroprevalence between first-time donors and repeat donors was a limitation to this study.

MAIN TEXT

INTRODUCTION

The global population is ageing as a combined result of the demographic transition from high to low levels of fertility and mortality.[1] Population ageing increases the total global disease burden, with approximately 23% attributable to disorders in people aged ≥60 years.[2] Chronic non-communicable diseases, including cardiovascular disease, malignant neoplasms, and chronic respiratory diseases, are the leading contributors to disease burden in older people.[2] However, infectious diseases also considerably affect older people, as an increasing incidence of infectious diseases, such as human immunodeficiency virus (HIV) and syphilis, was shown from recent surveillance data.[3-5] This large disease burden among older people calls for improvements in the healthcare system and more investments and programs focusing on healthy ageing.[2]

Syphilis, caused by *Treponema pallidum*, is a chronic infection with diverse clinical manifestations occurring in distinct stages, and may lead to blindness, dementia, delirium, death, etc., if not treated immediately or adequately.[6] Syphilis can also aid the passage for HIV to invade, reduce the CD4 T-cell levels, and increase the viral load, thereby aggravating the harm caused by HIV.[7] Even though syphilis can be effectively treated with penicillin, about 36.4 million new cases occur annually.[8] In China, the syphilis epidemic has rapidly increased, with a 16.3% increase per year during the first decade after the severe acute respiratory syndrome (SARS) outbreak.[9] The reported incidence was slightly higher among females than males (ratio, 1.00 to 0.92), but it varied significantly with age.[5] Younger people (aged 20-39 years) reported the highest syphilis incidence and accounted for the largest proportion of newly reported cases; however, the older age groups (aged ≥45 years) had the fastest growth in incidence, and males aged ≥60 years displayed a peak incidence of latent syphilis in the last decade.[5] With the accelerated ageing of the global population, the increasing syphilis epidemic among older adults is alarming.

Shenzhen, a special economic zone located in southern China and with a population

of >10 million, is one of the cities that most affected by syphilis. The reported incidence of syphilis was over 60 per 100 000 in last 10 years, which was much higher than the national incidence. [5,10] Consistent with the aforementioned characteristics of varied age groups, a rapid increase in syphilis incidence among older adults was observed in Shenzhen.[11] Studies usually considered blood donors as a representative of the general population and used the prevalence data of blood donors for real-time surveillance and identification of high-risk groups.[12] Whether the syphilis seroprevalence among blood donors agrees with reported incidence characteristics remains to be studied. Shenzhen launched a comprehensive program, the Shenzhen Program for Syphilis Prevention and Control (SPSPC), in November 2013 to enhance syphilis screening among blood donors and five other subgroups (HIV voluntary counsellors, methadone maintenance treatment users, female sex workers, men who have sex with men, and women of childbearing age), as well as case management, including diagnosis, treatment, and follow-up, for syphilis-infected adults.[13] Based on the data from the SPSPC, this study aimed to examine differences in syphilis seroprevalence among blood donors and describe the distribution of serological titres among syphilis-infected donors with respect to age groups, to confirm the syphilis epidemic characteristics in southern China and support the design of effective interventions for older adults.

METHODS

Subjects and blood donation process

Blood donors were recruited by the Shenzhen Blood Center between 2014 and 2017. About 10 blood mobiles, with the Shenzhen Blood Center logo and the words 'non-remunerated blood donation', were dispatched around the city to increase the accessibility of blood donation. Volunteers could either go to the mobiles or to the blood centre directly.

Before donation, all potential donors needed to complete a health history questionnaire, sign a donation registration form, and undergo rapid testing and a brief physical examination. The health history questionnaire contained a total of 27 medical conditions that would permanently or temporarily prevent the donors from

donation, including a series of chronic diseases and infectious diseases (e.g., HIV, syphilis), transplant, high-risk behaviours (e.g., homosexual behaviours, drug use), surgery, delivery, breastfeeding, etc. Pre-donation repaid testing included blood type, haemoglobin, hepatitis B surface antigen, and alanine transaminase. Weight, blood pressure, heart rate and body temperature were measured. Clinical examination of the skin and limbs was conducted. People who conformed to the Whole Blood and Component Donor Selection Requirements (GB 18467-2001) could proceed to donate blood. All blood donors were non-remunerated. Light refreshment, a blood donation certification, and a blood credit allowing free transfusion for donors or their direct relatives were provided as incentives. The donation process and blood management were fully in accordance with the *Blood Donation Law of the People's Republic of China* and the *Blood Donation Regulation of the Shenzhen Special Economic Zone*.

Serological testing

After donation, the blood samples were transferred to the Shenzhen Blood Center and underwent a series of laboratory testing. The enzyme-linked immunosorbent assay (ELISA; Zhuhai Lizhu Bio-engineering Co. Ltd., Zhuhai, China) was performed on all blood samples for syphilis screening. Syphilis-positive samples, with a form listing the donors' name, age, and gender, were then transferred to the Shenzhen Center for Chronic Disease Control [SZCCC, a city-level prevention and control centre for sexually transmitted diseases (STDs)] under SPSPC guidelines. A treponemal test of *Treponema pallidum* particle agglutination (TPPA; Fujirebio Inc., Tokyo, Japan) and a non-treponemal test of toluidine red unheated serum test (TRUST; Shanghai Rongsheng BioTech Co. Ltd., Shanghai, China) were used at the SZCCC to confirm the infection status. TRUST-positive samples further underwent quantitative titre testing to monitor response to treatment. TPPA and TRUST results were sent back to the Shenzhen Blood Center within 2 days after the samples were received.

Definition of syphilis infection

Based on serological test results, syphilis seropositivity was divided into historical infection and active infection, which was consistent with the classification from

previous studies.[12] Historical infection was defined as TPPA positive but TRUST negative and active infection as both TPPA and TRUST positive.[14] Syphilis seropositivity was defined as TPPA positive, including both TRUST negative and TRUST positive. Moreover, high-titre was defined as a quantitative titre of ≥ 1：8 in patients with active infection. For the purpose of this study, syphilis seropositivity which represented the overall infection status among the target population, and active infection and high-titre status which were correlated with disease activity, were analysed.

Statistical analysis

Primary outcomes of interest were the prevalence of syphilis seropositivity and active infection among all blood donors in different age groups. There were four age groups, <25 years, 25-34 years, 35-44 years, and ≥45 years, fully considering the age coverage of blood donors and age classification in previous studies.[15,16] We calculated the crude prevalence and its 95% confidence interval (CI). The chi-squared (χ^2) test for trend was used to assess the difference in prevalence among age groups. Odds ratios (ORs) and their 95% CIs were calculated when comparing the risk of syphilis seropositivity and active infection between the ≥ 45 years age group and other age groups. Line graphs were used to describe the changes in prevalence for both syphilis seropositivity and active infection among the age groups after stratification by gender and year of donation. Furthermore, we described the distribution of TRUST titres among the age groups and compared the difference using the χ^2 test for trend. Data were analysed using SPSS 17.0 for Windows (IBM Corp., Armonk, USA); $p < 0.05$ was considered statistically significant in the χ^2 test.

Patient and public involvement statement

Patients and the public were not involved in developing the hypothesis or research questions, nor were they involved in developing plans for the design or implementation of this study. The staff of the Shenzhen Blood Center were responsible for telling syphilis-positive participants about the test results and providing referral services related to syphilis treatment and management by phone.

RESULTS

Demographic characteristics

From 2014 to 2017, a total of 394 792 donors were recruited by the Shenzhen Blood Center for non-remunerated blood donation. Among them, 67.4% were male and 85.0% were aged <45 years. The distribution of age was varied between genders (χ^2 = 11249.0, p < 0.001) and among years of donation (χ^2 = 1182.0, p < 0.001). People aged 25-34 years accounted for the largest proportion of donors (**Table 1**).

Table 1 Characteristics of blood donors in different age groups in Shenzhen, 2014-2017

Variables	Aged <25 years (n=95736)	Aged 25-34 years (n=137447)	Aged 35-44 years (n=102422)	Aged ≥45 years (n=59187)	χ^2	p value
Gender					11249.0	<0.001
Male	51409(19.3%)	96237(36.2%)	74445(28.0%)	44061(16.6%)		
Female	44327(34.5%)	41210(32.0%)	27977(21.7%)	15126(11.8%)		
Year of donation					1182.0	<0.001
2014	22389(25.3%)	31929(36.0%)	23131(26.1%)	11210(12.6%)		
2015	24330(25.7%)	33096(35.0%)	24011(25.4%)	13241(14.0%)		
2016	24560(24.0%)	35736(34.9%)	26362(25.7%)	15843(15.5%)		
2017	24457(22.4%)	36686(33.7%)	28918(26.5%)	18893(17.3%)		

Prevalence of syphilis seropositivity and active infection

After ELISA testing, 2597 samples tested positive and were sent to the SZCCC for further examination. Among them, 733 (28.2%) were both TPPA and TRUST positive, 728 (28.0%) were only TPPA positive, and 1136 (43.7%) were false positive (**Figure 1**). The overall prevalence of syphilis seropositivity was 370.1 per 100 000 (95% CI, 351.1-389.0 per 100 000), and the prevalence of active infection was 185.7 per 100 000 (95% CI, 172.2-199.1 per 100 000). The prevalence of syphilis seropositivity and active infection was higher among females than males (syphilis seropositivity: χ^2 = 60.4, p < 0.001; active infection: χ^2 = 36.1, p < 0.001) and showed a decreasing trend from 2014 to 2017 (syphilis seropositivity: χ^2_{trend} = 27.1, p_{trend} < 0.001; active infection: χ^2_{trend} = 7.8, p_{trend} = 0.005). People aged ≥45 years reported the highest prevalence of both syphilis seropositivity and active infection, which was 3.8 times (OR = 3.8; 95% CI, 3.1-4.6) and 2.4 times (OR = 2.4; 95% CI, 1.9-3.0) higher than that among people

aged <25 years, and 2.3 times (OR = 2.3; 95% CI, 2.0-2.6) and 1.8 times (OR = 1.8; 95% CI, 1.5-2.2) higher than that among people aged 25-34 years, respectively. Trend analysis showed that the prevalence of syphilis seropositivity (χ^2_{trend} = 311.9, p_{trend} < 0.001) and active infection (χ^2_{trend} = 72.1, p_{trend} < 0.001) increased significantly with age (**Table 2**). After stratification by gender and year of donation, the increasing trend of prevalence with age remained (p_{trend} < 0.05), except for the prevalence of active infection among males (χ^2_{trend} = 0.923, p_{trend} = 0.337) and females (χ^2_{trend} = 0.224, p_{trend} = 0.636) in 2014 (**Figure 2**).

Table 2 Prevalence of syphilis seropositivity and active infection among blood donors in different age groups

Age group	Number of screened	Syphilis seropositivity				Active infection			
		Number	Prevalence per 100 000 (95%CI)	χ^2_{trend}	p_{trend} value	Number	Prevalence per 100 000 (95%CI)	χ^2_{trend}	p_{trend} value
Aged <25 years	95736	158	165.0(139.3-190.8)	311.9	<0.001	113	118.0(96.3-139.8)	72.1	<0.001
Aged 25-34 years	137447	376	273.6(245.9-301.2)			211	153.5(132.8-174.2)		
Aged 35-44 years	102422	559	545.8(500.7-590.9)			243	237.3(207.5-267.0)		
Aged ≥45 years	59187	368	621.8(558.4-685.1)			166	280.5(237.9-323.1)		

Distribution of TRUST titres

Among 733 donors with active infection, a TRUST titre of 1:1 accounted for the largest proportion (41.7%), followed by a titre of 1:2 (24.1%). About 27.0% had a TRUST titre of ≥1:8. The distribution of TRUST titres was varied among the age groups (**Figure 3**). Patients aged ≥45 years comprised a large proportion of low titres at 1:1 and 1:2, and the proportion of high titres was only 16.3%, which was much smaller than that among patients aged <25 years (51.3%) and 25-34 years (34.1%). The proportion of high-titre declined significantly with age (χ^2_{trend} = 53.6, p_{trend} < 0.001) (**Table 3**).

Table 3 Proportion of high-titre among active infection donors in different age groups

Age group	TRUST titre < 1：8	TRUST titre ≥ 1：8	χ^2_{trend}	p_{trend} value
Aged <25 years	55 (48.7%)	58 (51.3%)	53.6	<0.001
Aged 25-34 years	139 (65.9%)	72 (34.1%)		

Aged 35-44 years	202 (83.1%)	41 (16.9%)
Aged ≥45 years	139 (83.7%)	27 (16.3%)

DISCUSSION

This study identified that the overall prevalence of syphilis seropositivity among nearly 400 000 blood donors in 2014-2017 was 370.1 per 100 000, which was higher than that reported in the United States (54.6 per 100 000) and Brazil (135.5 per 100 000),[12,17] but lower than that reported in Ethiopia (732.4 per 100 000), Cameroon (3976.3 per 100 000), and India (1623.7 per 100 000).[18-20] The prevalence was similar to that in many cities in mainland China, such as Xi'an (359.6 per 100 000), Urumqi (359.3 per 100 000), and Kunming (381.2 per 100 000).[15,21] However, unlike some studies that used only one method (i.e., ELISA) to confirm the syphilis infection status and report the prevalence,[15,19,21,22] this study used ELISA as a screening test and then used TPPA and TRUST to confirm the serostatus if screened positive. As is known, ELISA is a method used worldwide for syphilis screening, with a sensitivity of >98% and specificity of >99% according to the reagent evaluation.[23] TPPA is considered as the gold standard test in syphilis diagnosis. Surprisingly, only 56.3% of ELISA-positive patients in this study were confirmed by TPPA, meaning the positive predictive value (the value associated with sensitivity, specificity and disease prevalence) for ELISA on syphilis was below 60% among blood donors. The testing process in this study greatly reduced the number of false positives and increased the accuracy of syphilis seroprevalence.

To our knowledge, this study is the first in-depth study focusing on active infection and serological titre distribution of syphilis among blood donors in mainland China. Active infection is different from historical infection as the former indicates more transmission and late syphilis if without timely and adequate treatment. The higher the serological titre, the more the risk of transmission (e.g., mother-to-child transmission) and adverse outcomes.[24] This study documented that 50.2% (733/1461) of syphilis seropositive donors had active infection, and 13.6% (198/1461) had a TRUST titre of ≥1:8. Here, the proportion of high titres among syphilis seropositive

patients was similar to that reported in the United States.[12]

This study found that syphilis prevalence significantly increased with age. Older adults aged ≥45 years displayed the highest prevalence of both syphilis seropositivity and active infection. More importantly, from the national surveillance data, people aged ≥60 years had a remarkably higher increase in reported incidence compared with those aged 45-60 years.[5] Hospitalised patients aged ≥70 years showed the highest syphilis prevalence (4.8%), followed by patients aged 61-70 years (3.9%) and those aged 51-60 years (3.2%), which was much different from that for HIV infection for which patients aged 31-40 years recorded the highest prevalence.[25] Based on the results of this study and previous studies, health awareness and syphilis prevention focusing on older adults are needed.

The higher prevalence among older adults might be due to several reasons. First, many older people are sexually active,[26] and their sexual health and behaviour affect syphilis transmission. Low self-perception of risk and misconceptions or limited knowledge about syphilis and other STDs were frequently reported as reasons for condomless sex among older adults.[5,27] Second, older adults have been largely neglected by healthcare providers due to age-related stigma.[28] Sexual health services for HIV or STDs rarely focus on older adults, leaving this group behind in both testing and prevention. Third, presenting with a late diagnosis has been significantly associated with older age. Older people were more likely to be aware of their serostatus when in hospital or had an active offer for testing.[29] In this study, analysis of the TRUST results suggests that > 90% of syphilis-infected people aged ≥45 years with TRUST negative or with low titres had a previous infection. However, late presentation is particularly worrying among older people because it further increases the risk of cardiovascular syphilis, neurosyphilis, paresis, etc. As syphilis is a great imitator, doctors often ignore syphilis infection when diagnosing the elderly, leading to omission of syphilis testing and misdiagnosis of the disease.

Evidence suggests that the most significant factor affecting testing patterns in older adults is the active provision of the screening test.[29] Since the initiation of China's

national syphilis control plan, syphilis screening has been widely integrated into HIV voluntary counselling and testing (VCT) services. More than 95% of people who received HIV testing services have undergone free syphilis testing.[30] Referral, treatment, and follow-up services would be provided to those diagnosed with syphilis. In Shenzhen, more than half of VCT sites are set in community health service centres, where a separate room is arranged for counselling and testing service. However, due to the low awareness of self-testing, older adults rarely positively seek the services. Meanwhile, most health staff are unwilling to provide the service actively because of limited experience, lack of time, discomfort in discussing sexual behaviours and STDs with older adults, stigma, ageism, etc.[28] Hence, enhanced training of healthcare providers and education of older adults are necessary.

Consistent with the results of some previous studies, the prevalence of both syphilis seropositivity and active infection were higher among females than males.[21,22] It may stem partly from the different physiology and anatomy of the genital organs between both sexes, leading to females being more likely to contract STDs in receptive vaginal sex behaviours.[31] Some studies have proved that the male-to-female transmission rate is higher than the female-to-male rate in certain STDs, such as HIV.[31,32] Besides, a proportion of females have multiple sex partners during their lifetime. A previous study has found that the syphilis prevalence among husbands of 2261 syphilis-infected pregnant women was < 30%.[33] Premarital or extramarital sexual partners may greatly increase the risk of syphilis infection among females. Additionally, serological response differs between males and females.[34] Females are more likely to be serofast [defined as remaining positive in a non-treponemal test and keeping the titre at a certain level (mostly 1:8 or below) after recommended therapy and follow-up 1 to 3 years according to syphilis stage] when comparing with males,[35] leading to more females staying in the state of active infection. The exact mechanism underlying this difference is unclear, but it may be partly associated with the varied immune system between both sexes.[34] Furthermore, men who have sex with men are considered a major high-risk subgroup for syphilis infection and are permanently deferred from blood donation in China.[36] In this study, males were excluded if they reported they had ever engaged in

homosexual behaviour in the health history questionnaire, which may be one of the reasons for the low syphilis prevalence among males.

Limitations

Our study has several limitations. First, limited financial and human resources restricted us in using a population-based design, which is considered as the gold standard in evaluating disease epidemics.[12] The choice of blood donors as population samples may result in potential bias, such as selection bias for age coverage and self-identified health conditions. Second, the syphilis seroprevalence among first-time donors was significantly higher than that among repeat donors.[12,15] This study did not collect the information of first-time donors and repeat donors, which may lead to underestimation of syphilis seroprevalence. Third, false-negative results attributable to the window period of syphilis infection may result in an underestimation of syphilis seroprevalence. However, the residual risk of syphilis infection is very low according to a residual risk analysis conducted in Shenzhen.[37]

Conclusions

This study provides an in-depth analysis of the association between syphilis seroprevalence and age. Older adults showed a high prevalence of both syphilis seropositivity and active infection but a small proportion of high titres, which point towards the compelling need to heighten awareness among healthcare providers and deliver more targeted prevention interventions for older adults to promote early testing.

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FIGURE LEGENDS

Figure 1 Flowchart for syphilis screening and confirmatory testing among blood donors. ELISA, enzyme-linked immunosorbent assay; TPPA, *Treponema pallidum* particle agglutination assay; TRUST, toluidine red unheated serum test.

Figure 2 Prevalence of syphilis seropositivity and active infection in different age groups, 2014-2017. (A) Prevalence of syphilis seropositivity and active infection among males. (B) Prevalence of syphilis seropositivity and active infection among females. SS, syphilis seropositivity; AI, active infection.

Figure 3 Distribution of TRUST titres among active infection donors in different age groups.

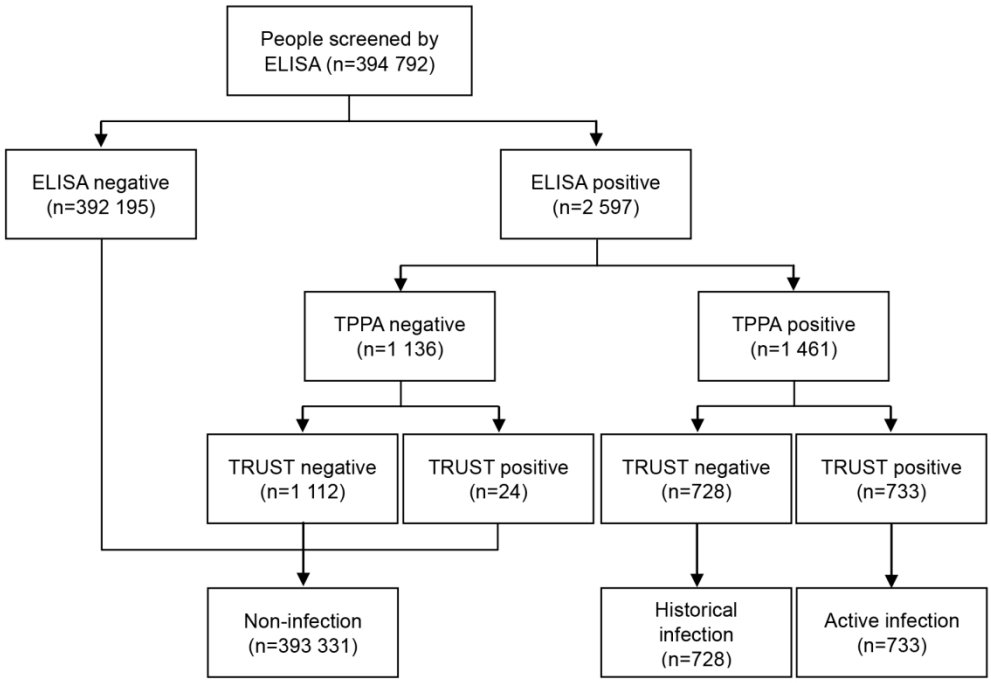
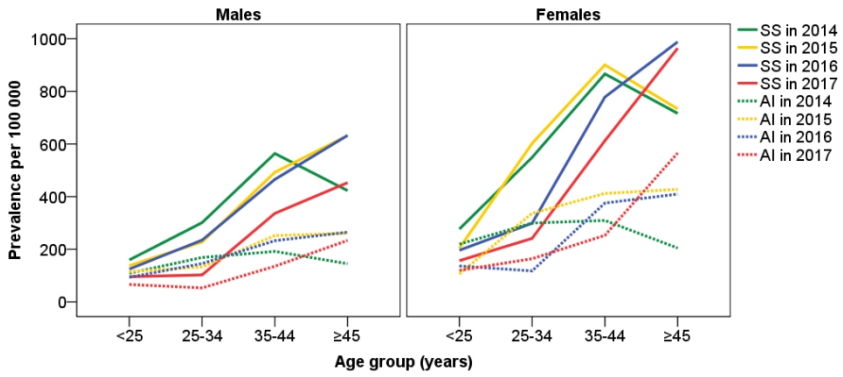


Figure 1 Flowchart for syphilis screening and confirmatory testing among blood donors. ELISA, enzyme-linked immunosorbent assay; TPPA, Treponema pallidum particle agglutination assay; TRUST, toluidine red unheated serum test.

157x116mm (300 x 300 DPI)



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424x182mm (72 x 72 DPI)

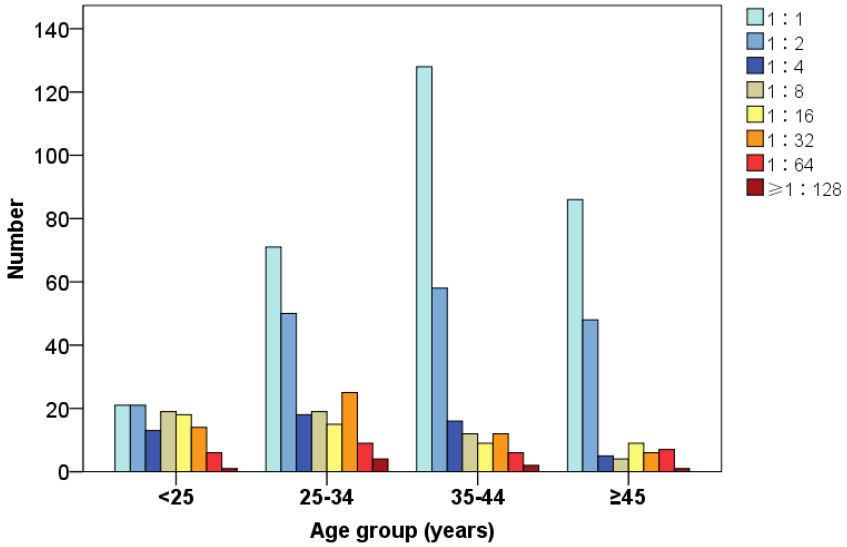

Figure 3 Distribution of TRUST titres among active infection donors in different age groups.

316x182mm (72 x 72 DPI)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	P1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	P6
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	P8-9
Objectives	3	State specific objectives, including any prespecified hypotheses	P9
Methods			
Study design	4	Present key elements of study design early in the paper	P9-10
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	P9-10
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	P9-10
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	P10-11
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	P10-11
Bias	9	Describe any efforts to address potential sources of bias	P9-10
Study size	10	Explain how the study size was arrived at	P9-10
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	P11
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	P11
		(*b*) Describe any methods used to examine subgroups and interactions	P11
		(*c*) Explain how missing data were addressed	Not applicable
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	Not applicable
		(*e*) Describe any sensitivity analyses	Not applicable
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	P12
		(b) Give reasons for non-participation at each stage	Not applicable
		(c) Consider use of a flow diagram	P23
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	P12
		(b) Indicate number of participants with missing data for each variable of interest	Not applicable
Outcome data	15*	Report numbers of outcome events or summary measures	P12-14
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	P12-14
		(*b*) Report category boundaries when continuous variables were categorized	P11
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	P12
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Not applicable
Discussion			
Key results	18	Summarise key results with reference to study objectives	P14
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	P17
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	P14-17
Generalisability	21	Discuss the generalisability (external validity) of the study results	P17
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	P18

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Title page

Association between syphilis seroprevalence and age among blood donors in southern China: an observational study from 2014 to 2017

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ABSTRACT

Objective: This study investigated the association between syphilis seroprevalence and age among blood donors, and described the distribution of serological titres among syphilis-infected donors, aiming to confirm the syphilis epidemic characteristics and to promote effective interventions for older adults.

Methods: Data were obtained from the Shenzhen Program for Syphilis Prevention and Control in 2014-2017. Blood samples were screened using the enzyme-linked immunosorbent assays (ELISAs), and confirmed using the *Treponema pallidum* particle agglutination assay (TPPA) and toluidine red unheated serum test (TRUST).

Results: Among 394 792 blood donors, 733 tested TPPA and TRUST positive (active infection), and 728 tested only TPPA positive (historical infection). The overall prevalence of syphilis seropositivity was 370.1 per 100 000 [95% confidence interval (CI), 351.1-389.0 per 100 000]; the prevalence of active infection was 185.7 per 100 000 (95% CI, 172.2-199.1 per 100 000). People aged ≥45 years displayed a prevalence of 621.8 per 100 000 in syphilis seropositivity and 280.5 per 100 000 in active infection, which were 3.8 times and 2.4 times higher than that for people aged <25 years, respectively. The prevalence of syphilis seropositivity (χ^2_{trend} = 311.9, p_{trend} < 0.001) and active infection (χ^2_{trend} = 72.1, p_{trend} < 0.001) increased significantly with age. After stratification by gender and year of donation , the increasing trend of prevalence with age remained(p_{trend} < 0.05), except for the prevalence of active infection in males and females in 2014. About 16.3% of donors with active infection and aged ≥45 years had a TRUST titre of ≥1:8, lower than that of patients aged <25 years (51.3%) and 25-34 years (34.1%).

Conclusions: The findings confirm the high prevalence of syphilis among older adults, and suggest the need to increase awareness among healthcare providers and deliver more targeted prevention interventions for older adults to promote early testing.

Strengths and limitations of this study

- This study described the syphilis seroprevalence among nearly 400 000 blood donors, including syphilis seropositivity, active infection, and distribution of serological titres.
- The testing process in this study, using enzyme-linked immunosorbent assays (ELISAs) as a screening test and then using *Treponema pallidum* particle agglutination assay (TPPA) and toluidine red unheated serum test (TRUST) to confirm the serostatus, increased the accuracy of syphilis seroprevalence.
- Lack of information on syphilis seroprevalence between first-time donors and repeat donors was a limitation to this study.

MAIN TEXT

INTRODUCTION

The global population is ageing as a combined result of the demographic transition from high to low levels of fertility and mortality.[1] Population ageing increases the total global disease burden, with approximately 23% attributable to disorders in people aged ≥60 years.[2] Chronic non-communicable diseases, including cardiovascular disease, malignant neoplasms, and chronic respiratory diseases, are the leading contributors to disease burden in older people.[2] However, infectious diseases also considerably affect older people, as an increasing incidence of infectious diseases, such as human immunodeficiency virus (HIV) and syphilis, was shown from recent surveillance data.[3-5] This large disease burden among older people calls for improvements in the healthcare system and more investments and programs focusing on healthy ageing.[2]

Syphilis, caused by *Treponema pallidum*, is a chronic infection with diverse clinical manifestations occurring in distinct stages, and may lead to blindness, dementia, delirium, death, etc., if not treated immediately or adequately.[6] Syphilis can also aid the passage for HIV to invade, reduce the CD4 T-cell levels, and increase the viral load, thereby aggravating the harm caused by HIV.[7] Even though syphilis can be effectively treated with penicillin, about 36.4 million new cases occur annually.[8] In China, the syphilis epidemic has rapidly increased, with a 16.3% increase per year during the first decade after the severe acute respiratory syndrome (SARS) outbreak.[9] The reported incidence was slightly higher among females than males (ratio, 1.00 to 0.92), but it varied significantly with age.[5] Younger people (aged 20-39 years) reported the highest syphilis incidence and accounted for the largest proportion of newly reported cases; however, the older age groups (aged ≥45 years) had the fastest growth in incidence, and males aged ≥60 years displayed a peak incidence of latent syphilis in the last decade.[5] With the accelerated ageing of the global population, the increasing syphilis epidemic among older adults is alarming.

Shenzhen, a special economic zone located in southern China and with a population

of >10 million, is one of the cities that most affected by syphilis. The reported incidence of syphilis was over 60 per 100 000 in last 10 years, which was much higher than the national incidence. [5,10] Consistent with the aforementioned characteristics of varied age groups, a rapid increase in syphilis incidence among older adults was observed in Shenzhen.[11] Studies usually considered blood donors as a representative of the general population and used the prevalence data of blood donors for real-time surveillance and identification of high-risk groups.[12] Whether the syphilis seroprevalence among blood donors agrees with reported incidence characteristics remains to be studied. Shenzhen launched a comprehensive program, the Shenzhen Program for Syphilis Prevention and Control (SPSPC), in November 2013 to enhance syphilis screening among blood donors and five other subgroups (HIV voluntary counsellors, methadone maintenance treatment users, female sex workers, men who have sex with men, and women of childbearing age), as well as case management, including diagnosis, treatment, and follow-up, for syphilis-infected adults.[13] Based on the data from the SPSPC, this study aimed to examine differences in syphilis seroprevalence among blood donors and describe the distribution of serological titres among syphilis-infected donors with respect to age groups, to confirm the syphilis epidemic characteristics in southern China and support the design of effective interventions for older adults.

METHODS

Subjects and blood donation process

Blood donors were recruited by the Shenzhen Blood Center between 2014 and 2017. About 10 blood mobiles, with the Shenzhen Blood Center logo and the words 'non-remunerated blood donation', were dispatched around the city to increase the accessibility of blood donation. Volunteers could either go to the mobiles or to the blood centre directly.

Before donation, all potential donors needed to sign a donation registration form, complete a health history questionnaire, and undergo rapid testing and a brief physical examination. A concise introduction of blood use, donation procedure, laboratory testing, and legal and regulatory requirements was shown at the front of

the registration form. Inform consent was obtained from the donors for the laboratory testing and use of the blood, the academic use of the data and the publication of the report. The health history questionnaire contained a total of 27 medical conditions that would permanently or temporarily prevent the donors from donation, including a series of chronic diseases and infectious diseases (e.g., HIV, syphilis), transplant, high-risk behaviours (e.g., homosexual behaviours, drug use), surgery, delivery, breastfeeding, etc. Pre-donation repaid testing included blood type, haemoglobin, hepatitis B surface antigen, and alanine transaminase. Weight, blood pressure, heart rate and body temperature were measured. Clinical examination of the skin and limbs was conducted. People who conformed to the *Whole Blood and Component Donor Selection Requirements (GB 18467-2001)* could proceed to donate blood. All blood donors were non-remunerated. Light refreshment, a blood donation certification, and a blood credit allowing free transfusion for donors or their direct relatives were provided as incentives. The donation process and blood management were fully in accordance with the *Blood Donation Law of the People's Republic of China* and the *Blood Donation Regulation of the Shenzhen Special Economic Zone*.

Serological testing

After donation, the blood samples were transferred to the Shenzhen Blood Center and underwent a series of laboratory testing. The enzyme-linked immunosorbent assays (ELISAs) with two different reagents (Zhuhai Lizhu Bio-engineering Co. Ltd., Zhuhai, China; DiaSorin S.p.A. UK Branch, UK) were performed simultaneously on all blood samples for syphilis screening. Syphilis-positive samples of one or both screening assays, with a form listing the donors' name, age, and gender, were then transferred to the Shenzhen Center for Chronic Disease Control [SZCCC, a city-level prevention and control centre for sexually transmitted diseases (STDs)] under SPSPC guidelines. A treponemal test of *Treponema pallidum* particle agglutination (TPPA; Fujirebio Inc., Tokyo, Japan) and a non-treponemal test of toluidine red unheated serum test (TRUST; Shanghai Rongsheng BioTech Co. Ltd., Shanghai, China) were used at the SZCCC to confirm the infection status. TRUST-positive samples further underwent quantitative titre testing to monitor response to treatment. TPPA and TRUST results were sent back to the Shenzhen Blood Center within 2 days after the

samples were received.

Definition of syphilis infection

Based on serological test results, syphilis seropositivity was divided into historical infection and active infection, which was consistent with the classification from previous studies.[12] Historical infection was defined as TPPA positive but TRUST negative and active infection as both TPPA and TRUST positive.[14] Syphilis seropositivity was defined as TPPA positive, including both TRUST negative and TRUST positive. Moreover, high-titre was defined as a quantitative titre of ≥ 1：8 in patients with active infection. For the purpose of this study, syphilis seropositivity which represented the overall infection status among the target population, and active infection and high-titre status which were correlated with disease activity, were analysed.

Statistical analysis

Data of donors' number among different subgroups (age, gender and year of donation) and syphilis testing results were sourced from the Shenzhen Blood Center and the SZCCC, respectively. Primary outcomes of interest were the prevalence of syphilis seropositivity and active infection among all blood donors in different age groups. There were four age groups, <25 years, 25-34 years, 35-44 years, and ≥45 years, fully considering the age coverage of blood donors and age classification in previous studies.[15,16] We calculated the crude prevalence and its 95% confidence interval (CI). The chi-squared (χ^2) test for trend was used to assess the difference in prevalence among age groups. Odds ratios (ORs) and their 95% CIs were calculated when comparing the risk of syphilis seropositivity and active infection between the ≥ 45 years age group and other age groups. Line graphs were used to describe the changes in prevalence for both syphilis seropositivity and active infection among the age groups after stratification by gender and year of donation. Furthermore, we described the distribution of TRUST titres among the age groups and compared the difference using the χ^2 test for trend. Data were analysed using SPSS 17.0 for Windows (IBM Corp., Armonk, USA); $p < 0.05$ was considered statistically significant in the χ^2 test.

Patient and public involvement statement

Patients and the public were not involved in developing the hypothesis or research questions, nor were they involved in developing plans for the design or implementation of this study. The staff of the Shenzhen Blood Center were responsible for telling syphilis-positive participants about the test results and providing referral services related to syphilis treatment and management by phone.

RESULTS

Demographic characteristics

From 2014 to 2017, a total of 394 792 donors were recruited by the Shenzhen Blood Center for non-remunerated blood donation. Among them, 67.4% were male and 85.0% were aged <45 years. The distribution of age was varied between genders (χ^2 = 11249.0, $p < 0.001$) and among years of donation (χ^2 = 1182.0, $p < 0.001$). People aged 25-34 years accounted for the largest proportion of donors (**Table 1**).

Table 1 Characteristics of blood donors in different age groups in Shenzhen, 2014-2017

Variables	Aged <25 years (n=95736)	Aged 25-34 years (n=137447)	Aged 35-44 years (n=102422)	Aged ≥45 years (n=59187)	χ^2	*p* value
Gender					11249.0	<0.001
Male	51409(19.3%)	96237(36.2%)	74445(28.0%)	44061(16.6%)		
Female	44327(34.5%)	41210(32.0%)	27977(21.7%)	15126(11.8%)		
Year of donation					1182.0	<0.001
2014	22389(25.3%)	31929(36.0%)	23131(26.1%)	11210(12.6%)		
2015	24330(25.7%)	33096(35.0%)	24011(25.4%)	13241(14.0%)		
2016	24560(24.0%)	35736(34.9%)	26362(25.7%)	15843(15.5%)		
2017	24457(22.4%)	36686(33.7%)	28918(26.5%)	18893(17.3%)		

Prevalence of syphilis seropositivity and active infection

After ELISA testing, 2597 samples tested positive and were sent to the SZCCC for further examination. Among them, 733 (28.2%) were both TPPA and TRUST positive, 728 (28.0%) were only TPPA positive, and 1136 (43.7%) were false positive (**Figure 1**). The overall prevalence of syphilis seropositivity was 370.1 per 100 000 (95% CI,

351.1-389.0 per 100 000), and the prevalence of active infection was 185.7 per 100 000 (95% CI, 172.2-199.1 per 100 000). The prevalence of syphilis seropositivity and active infection was higher among females than males (syphilis seropositivity: χ^2 = 60.4, $p < 0.001$; active infection: χ^2 = 36.1, $p < 0.001$) and showed a decreasing trend from 2014 to 2017 (syphilis seropositivity: χ^2_{trend} = 27.1, $p_{trend} < 0.001$; active infection: χ^2_{trend} = 7.8, p_{trend} = 0.005). People aged ≥45 years reported the highest prevalence of both syphilis seropositivity and active infection, which was 3.8 times (OR = 3.8; 95% CI, 3.1-4.6) and 2.4 times (OR = 2.4; 95% CI, 1.9-3.0) higher than that among people aged <25 years, and 2.3 times (OR = 2.3; 95% CI, 2.0-2.6) and 1.8 times (OR = 1.8; 95% CI, 1.5-2.2) higher than that among people aged 25-34 years, respectively. Trend analysis showed that the prevalence of syphilis seropositivity (χ^2_{trend} = 311.9, $p_{trend} <$ 0.001) and active infection (χ^2_{trend} = 72.1, $p_{trend} < 0.001$) increased significantly with age (**Table 2**). After stratification by gender and year of donation, the increasing trend of prevalence with age remained ($p_{trend} < 0.05$), except for the prevalence of active infection among males (χ^2_{trend} = 0.923, p_{trend} = 0.337) and females (χ^2_{trend} = 0.224, p_{trend} = 0.636) in 2014 (**Figure 2**).

Table 2 Prevalence of syphilis seropositivity and active infection among blood donors in different age groups

Age group	Number of screened	Syphilis seropositivity				Active infection			
		Number	Prevalence per 100 000 (95%CI)	χ^2_{trend}	p_{trend} value	Number	Prevalence per 100 000 (95%CI)	χ^2_{trend}	p_{trend} value
Aged <25 years	95736	158	165.0(139.3-190.8)	311.9	<0.001	113	118.0(96.3-139.8)	72.1	<0.001
Aged 25-34 years	137447	376	273.6(245.9-301.2)			211	153.5(132.8-174.2)		
Aged 35-44 years	102422	559	545.8(500.7-590.9)			243	237.3(207.5-267.0)		
Aged ≥45 years	59187	368	621.8(558.4-685.1)			166	280.5(237.9-323.1)		

Distribution of TRUST titres

Among 733 donors with active infection, a TRUST titre of 1:1 accounted for the largest proportion (41.7%), followed by a titre of 1:2 (24.1%). About 27.0% had a TRUST titre of ≥1:8. The distribution of TRUST titres was varied among the age groups (**Figure 3**). Patients aged ≥45 years comprised a large proportion of low titres at 1:1 and 1:2, and the proportion of high titres was only 16.3%, which was much

smaller than that among patients aged <25 years (51.3%) and 25-34 years (34.1%). The proportion of high-titre declined significantly with age (χ^2_{trend} = 53.6, p_{trend} < 0.001) (**Table 3**).

Table 3 Proportion of high-titre among active infection donors in different age groups

Age group	TRUST titre < 1∶8	TRUST titre ≥ 1∶8	χ^2_{trend}	p_{trend} value
Aged <25 years	55 (48.7%)	58 (51.3%)	53.6	<0.001
Aged 25-34 years	139 (65.9%)	72 (34.1%)		
Aged 35-44 years	202 (83.1%)	41 (16.9%)		
Aged ≥45 years	139 (83.7%)	27 (16.3%)		

DISCUSSION

This study identified that the overall prevalence of syphilis seropositivity among nearly 400 000 blood donors in 2014-2017 was 370.1 per 100 000, which was higher than that reported in the United States (54.6 per 100 000) and Brazil (135.5 per 100 000),[12,17] but lower than that reported in Ethiopia (732.4 per 100 000), Cameroon (3976.3 per 100 000), and India (1623.7 per 100 000).[18-20] The prevalence was similar to that in many cities in mainland China, such as Xi'an (359.6 per 100 000), Urumqi (359.3 per 100 000), and Kunming (381.2 per 100 000).[15,21] However, unlike some studies that used only one method (i.e., ELISA) to confirm the syphilis infection status and report the prevalence,[15,19,21,22] this study used ELISA as a screening test and then used TPPA and TRUST to confirm the serostatus if screened positive. As is known, ELISA is a method used worldwide for syphilis screening, with a sensitivity of >95% and specificity of >99% according to the reagent evaluation.[23] TPPA is considered as the gold standard test in syphilis diagnosis. Surprisingly, only 56.3% of ELISA-positive patients in this study were confirmed by TPPA, meaning the positive predictive value (the value associated with sensitivity, specificity and disease prevalence) for ELISA on syphilis was below 60% among blood donors. The testing process in this study greatly reduced the number of false positives and increased the accuracy of syphilis seroprevalence.

To our knowledge, this study is the first in-depth study focusing on active infection and serological titre distribution of syphilis among blood donors in mainland China. Active infection is different from historical infection as the former indicates more transmission and late syphilis if without timely and adequate treatment. The higher the serological titre, the more the risk of transmission (e.g., mother-to-child transmission) and adverse outcomes.[24] This study documented that 50.2% (733/1461) of syphilis seropositive donors had active infection, and 13.6% (198/1461) had a TRUST titre of ≥1:8. Here, the proportion of high titres among syphilis seropositive patients was similar to that reported in the United States.[12]

This study found that syphilis prevalence significantly increased with age. Older adults aged ≥45 years displayed the highest prevalence of both syphilis seropositivity and active infection. More importantly, from the national surveillance data, people aged ≥60 years had a remarkably higher increase in reported incidence compared with those aged 45-60 years.[5] Hospitalised patients aged ≥70 years showed the highest syphilis prevalence (4.8%), followed by patients aged 61-70 years (3.9%) and those aged 51-60 years (3.2%), which was much different from that for HIV infection for which patients aged 31-40 years recorded the highest prevalence.[25] Based on the results of this study and previous studies, health awareness and syphilis prevention focusing on older adults are needed.

The higher prevalence among older adults might be due to several reasons. First, many older people are sexually active,[26] and their sexual health and behaviour affect syphilis transmission. Low self-perception of risk and misconceptions or limited knowledge about syphilis and other STDs were frequently reported as reasons for condomless sex among older adults.[5,27] Second, older adults have been largely neglected by healthcare providers due to age-related stigma.[28] Sexual health services for HIV or STDs rarely focus on older adults, leaving this group behind in both testing and prevention. Third, presenting with a late diagnosis has been significantly associated with older age. Older people were more likely to be aware of their serostatus when in hospital or had an active offer for testing.[29] In this study, analysis of the TRUST results suggests that > 90% of syphilis-infected people aged ≥45 years

with TRUST negative or with low titres had a previous infection. However, late presentation is particularly worrying among older people because it further increases the risk of cardiovascular syphilis, neurosyphilis, paresis, etc. As syphilis is a great imitator, doctors often ignore syphilis infection when diagnosing the elderly, leading to omission of syphilis testing and misdiagnosis of the disease.

Evidence suggests that the most significant factor affecting testing patterns in older adults is the active provision of the screening test.[29] Since the initiation of China's national syphilis control plan, syphilis screening has been widely integrated into HIV voluntary counselling and testing (VCT) services. More than 95% of people who received HIV testing services have undergone free syphilis testing.[30] Referral, treatment, and follow-up services would be provided to those diagnosed with syphilis. In Shenzhen, more than half of VCT sites are set in community health service centres, where a separate room is arranged for counselling and testing service. However, due to the low awareness of self-testing, older adults rarely positively seek the services. Meanwhile, most health staff are unwilling to provide the service actively because of limited experience, lack of time, discomfort in discussing sexual behaviours and STDs with older adults, stigma, ageism, etc.[28] Hence, enhanced training of healthcare providers and education of older adults are necessary.

Consistent with the results of some previous studies, the prevalence of both syphilis seropositivity and active infection were higher among females than males.[21,22] It may stem partly from the different physiology and anatomy of the genital organs between both sexes, leading to females being more likely to contract STDs in receptive vaginal sex behaviours.[31] Some studies have proved that the male-to-female transmission rate is higher than the female-to-male rate in certain STDs, such as HIV.[31,32] Besides, a proportion of females have multiple sex partners during their lifetime. A previous study has found that the syphilis prevalence among husbands of 2261 syphilis-infected pregnant women was < 30%.[33] Premarital or extramarital sexual partners may greatly increase the risk of syphilis infection among females. Additionally, serological response differs between males and females.[34] Females are more likely to be serofast [defined as remaining positive in a

non-treponemal test and keeping the titre at a certain level (mostly 1:8 or below) after recommended therapy and follow-up 1 to 3 years according to syphilis stage] when comparing with males,[35] leading to more females staying in the state of active infection. The exact mechanism underlying this difference is unclear, but it may be partly associated with the varied immune system between both sexes.[34] Furthermore, men who have sex with men are considered a major high-risk subgroup for syphilis infection and are permanently deferred from blood donation in China.[36] In this study, males were excluded if they reported they had ever engaged in homosexual behaviour in the health history questionnaire, which may be one of the reasons for the low syphilis prevalence among males.

Limitations

Our study has several limitations. First, limited financial and human resources restricted us in using a population-based design, which is considered as the gold standard in evaluating disease epidemics.[12] The choice of blood donors as population samples may result in potential bias, such as selection bias for age coverage and self-identified health conditions. Second, the syphilis seroprevalence among first-time donors was significantly higher than that among repeat donors.[12,15] This study did not collect the information of first-time donors and repeat donors, which may lead to underestimation of syphilis seroprevalence. Third, false-negative results attributable to the window period of syphilis infection may result in an underestimation of syphilis seroprevalence. However, the residual risk of syphilis infection is very low according to a residual risk analysis conducted in Shenzhen.[37] Fourth, this study used two reagents in syphilis screening. Samples with one positive result or both positive results would be considered as problematic samples. This parallel testing method was strict and suitable for blood donors. However, we did not collect the data of each reagent and the positive predictive value cannot be calculated respectively.

Conclusions

This study provides an in-depth analysis of the association between syphilis seroprevalence and age. Older adults showed a high prevalence of both syphilis

seropositivity and active infection but a small proportion of high titres, which point towards the compelling need to heighten awareness among healthcare providers and deliver more targeted prevention interventions for older adults to promote early testing.

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Contributors XW and TF contributed to designing the study, coordinating data collection and drafting the article. YG, JY, CZ and FT contributed to data collection, patient treatment and disease management. HF, LL and FW contributed to data collection and data analysis. FW contributed to syphilis testing and laboratory quality control. YC and WY contributed to making important comments of the manuscript. All authors read and approved the final draft of the manuscript.

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Competing interests The authors declare no competing interest.

Patient consent Not applicable.

Ethics approval Ethics approval was obtained from the Ethics Committee of Shenzhen Center for Chronic Disease Control (No. 20180212).

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement Data are available by emailing XW(bingfsh@126.com).

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FIGURE LEGENDS

Figure 1 Flowchart for syphilis screening and confirmatory testing among blood donors. ELISA, enzyme-linked immunosorbent assay; TPPA, *Treponema pallidum* particle agglutination assay; TRUST, toluidine red unheated serum test.

Figure 2 Prevalence of syphilis seropositivity and active infection in different age groups, 2014-2017. (A) Prevalence of syphilis seropositivity and active infection among males. (B) Prevalence of syphilis seropositivity and active infection among females. SS, syphilis seropositivity; AI, active infection.

Figure 3 Distribution of TRUST titres among active infection donors in different age groups.

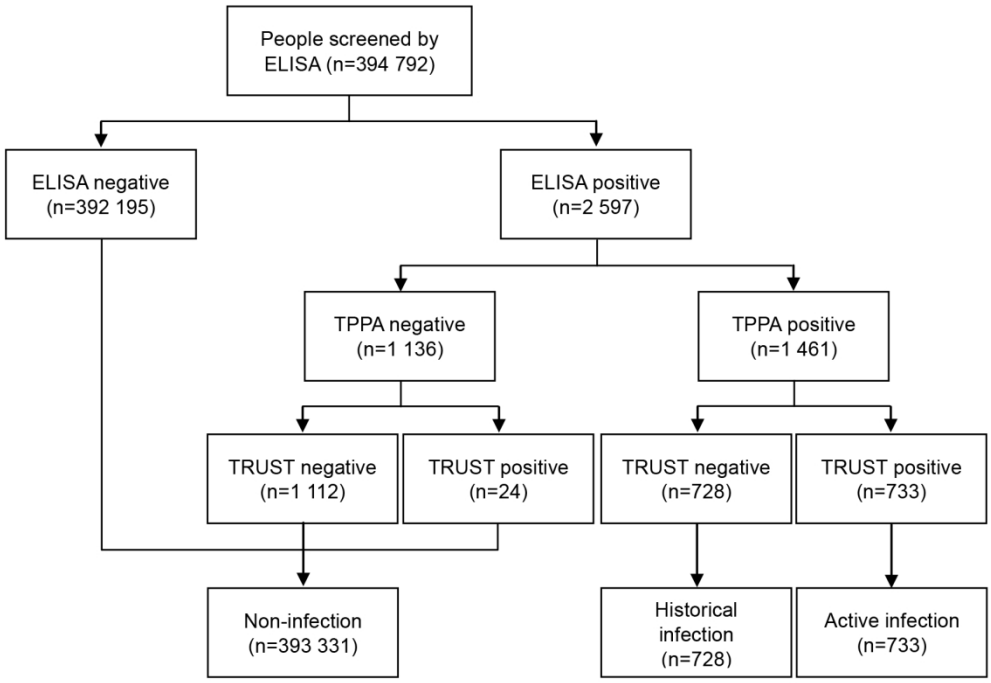

Figure 1 Flowchart for syphilis screening and confirmatory testing among blood donors. ELISA, enzyme-linked immunosorbent assay; TPPA, Treponema pallidum particle agglutination assay; TRUST, toluidine red unheated serum test.

157x116mm (300 x 300 DPI)

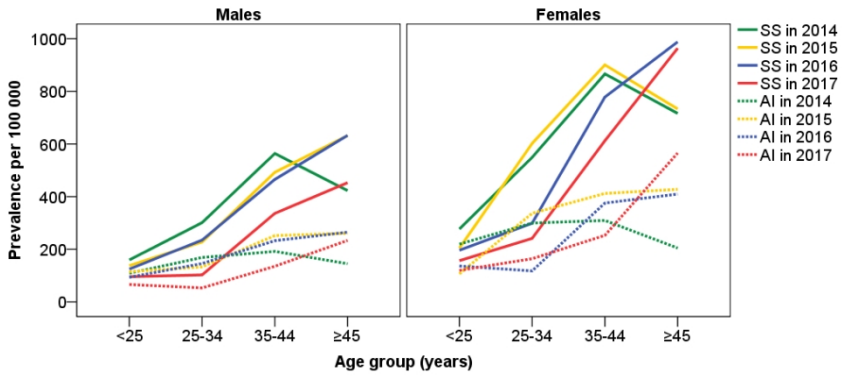

Figure 2 Prevalence of syphilis seropositivity and active infection in different age groups, 2014-2017. (A) Prevalence of syphilis seropositivity and active infection among males. (B) Prevalence of syphilis seropositivity and active infection among females. SS, syphilis seropositivity; AI, active infection.

424x182mm (72 x 72 DPI)

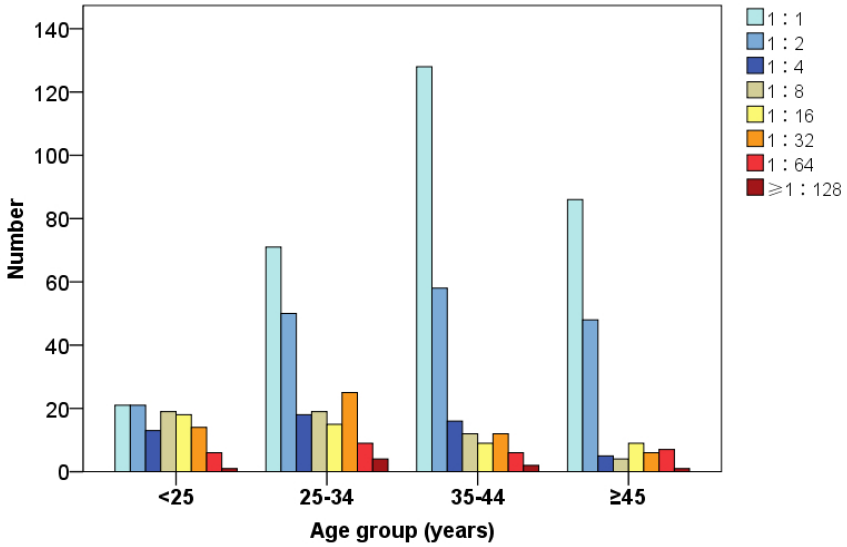

Figure 3 Distribution of TRUST titres among active infection donors in different age groups.

316x182mm (72 x 72 DPI)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	P1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	P6
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	P8-9
Objectives	3	State specific objectives, including any prespecified hypotheses	P9
Methods			
Study design	4	Present key elements of study design early in the paper	P9-10
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	P9-10
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	P9-10
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	P10-11
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	P10-11
Bias	9	Describe any efforts to address potential sources of bias	P9-10
Study size	10	Explain how the study size was arrived at	P9-10
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	P11
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	P11
		(*b*) Describe any methods used to examine subgroups and interactions	P11
		(*c*) Explain how missing data were addressed	Not applicable
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	Not applicable
		(*e*) Describe any sensitivity analyses	Not applicable
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	P12
		(b) Give reasons for non-participation at each stage	Not applicable
		(c) Consider use of a flow diagram	P23
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	P12
		(b) Indicate number of participants with missing data for each variable of interest	Not applicable
Outcome data	15*	Report numbers of outcome events or summary measures	P12-14
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	P12-14
		(*b*) Report category boundaries when continuous variables were categorized	P11
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	P12
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Not applicable
Discussion			
Key results	18	Summarise key results with reference to study objectives	P14
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	P17
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	P14-17
Generalisability	21	Discuss the generalisability (external validity) of the study results	P17
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	P18

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.