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Original Article

Clinical analysis and methodological evaluation of syphilis infection in patients in a first-class tertiary hospital in Suzhou, China

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Abstract: Objective: To explore the distribution and epidemiological characteristics of patients with syphilis in a first-class tertiary hospital and to evaluate the coincidence rate between chemiluminescence immunoassay (CLIA) and *Treponema pallidum* particle agglutination assay (TPPA). Methods: The medical records of 247,501 outpatients and inpatients were retrospectively analyzed. TPPA was used to verify positive and suspected cases, and the coincidence rate between CLIA and TPPA was evaluated. Receiver operating characteristic (ROC) curve was used to determine optimal diagnostic thresholds. Results: Of the 247,501 serum samples, 5,173 were detected positive for syphilis using CLIA, with a detection rate of 2.09% and a men-to-women ratio of 1.39. The chi-square test showed that sex and age were both factors that affected the detection rate (χ^2=229.51, P < 0.0001). In addition, urology, orthopedics, cardiology, general surgery, gastroenterology, and gynecology represented the top six departments with the highest numbers of positive cases. Comparative analysis showed that the overall coincidence rate between CLIA and TPPA was 80.24%. Analysis of the ROC curve showed that the area under the curve (AUC) was 0.936 (95% confidence interval [CI]: 0.929-0.942, P < 0.0001) using sample/cut-off value (S/CO) as a diagnostic indicator. The results showed that an S/CO value of 3.945 was the best diagnostic value for the CLIA method, with a diagnostic specificity of 93.64% and a sensitivity of 81.90%. Conclusions: Syphilis is widely distributed in various hospital departments and primarily affects middle-aged and older individuals. For cases that have been initially screened as positive or suspicious, TPPA and other tests should be used for verification to avoid misdiagnosis and missed diagnosis.

Keywords: Syphilis, epidemiology, serological screening, diagnostic performance

Introduction

Syphilis is a sexually transmitted disease (STD) caused by *Treponema pallidum* subsp. *pallidum*. Except for fetal infections that cause congenital syphilis, other infections are mainly transmitted through sexual contact. Although low-cost antibiotics are sufficient to treat syphilis, it remains a high-risk disease that causes undesirable obstetric outcomes and increases HIV transmission [1]. Syphilis represents a long-standing threat to global public health, with approximately 6.3 million newly diagnosed cases worldwide every year [2].

Since the beginning of the 21st century, syphilis has caused an increasing healthcare burden in China. In 2017, 534,622 cases of syphilis were reported nationwide, an increase of 8.4% compared to 2016 (493,026 cases) [3]. In 2016, the 69th World Health Assembly set the goal of reducing the global incidence of syphilis by 90% from 2018 to 2030 [4]. In response to this WHO initiative, the Chinese Ministry of Health launched China's National Syphilis Prevention and Control Program in June 2010, with the aim of reducing primary and secondary syphilis, as well as eliminating congenital syphilis. This means that after 2020, there will be <

15 syphilis cases per 100,000 people in China [5].

Jiangsu Province, located in the eastern coastal area of China, is one of the provinces with a high prevalence of syphilis. According to statistics from China's Information System for Disease Control and Prevention, syphilis has been the third most frequently reported infectious disease in Jiangsu since 2007, with approximately 24,000 cases reported every year [6]. Suzhou is the city with the largest permanent population in the Jiangsu Province. This large population has also brought many challenges to the prevention of STDs such as syphilis. Therefore, a clear understanding of the epidemiological characteristics of syphilis cases in Jiangsu and the selection of appropriate testing methods are particularly important for the treatment and eradication of syphilis.

Diagnosis of syphilis traditionally includes non-specific non-treponemal serology for lipid antigens such as the rapid plasma regain (RPR) test, specific treponemal tests such as the *Treponema pallidum particle agglutination assay* (TPPA), and the fluorescent treponemal antibody-absorbed (FTA-ABS) test [7]. There are two main syphilis testing algorithms: "traditional" and "reverse" algorithms. Because the former uses low-sensitivity non-treponemal detection, there is a risk of underdiagnosis [8]. Therefore, the "reverse algorithm" is generally used in clinical practice, which uses treponemal detection followed by non-treponemal detection for reactive samples. The TPPA is currently recognized as a confirmatory test due to its high sensitivity and specificity [9]. Chemiluminescence immunoassay (CLIA) has been widely used in the screening of clinical syphilis-specific antibodies due to its high throughput and simple operation; however, its false-positive rate cannot be ignored [10, 11].

In this study, the TPPA method was used as a confirmatory test to calculate the positive predictive value (PPV) of CLIA. The most suitable cut-off value was established through methodological evaluation, and an epidemiological survey was conducted on 247,501 screened individuals, in an effort to provide better direction for the selection of syphilis prevention and detection strategies in China.

Materials and methods

Data collection

Data were obtained from syphilis antibody tests performed at the Center of Clinical Laboratory of the First Affiliated Hospital of Soochow University (Suzhou, China) between January 2019 and August 2021. A total of 247,501 outpatient and inpatient cases were included.

Instruments and reagents

All samples were analyzed using CLIA using the Architect Syphilis TP system (Abbott i2000SR, Wiesbaden, Germany). The kit contained paramagnetic microparticles coated with recombinant TpN15, TpN17, and TpN47 antigens. If the serum sample contains antibodies against *T. pallidum*, it is absorbed by paramagnetic microparticles during the incubation process and coupled with acridinium-labeled anti-human IgG or IgM conjugates. This process produces a chemiluminescence reaction, and the relative light units (RLUs) obtained are positively correlated with anti-*T. pallidum* antibodies in the samples. The RLU of the sample/cut-off value (S/CO) is used as the unit of the result, where a ratio ≥ 1 is considered positive and a ratio between 0.9 and 1 is considered suspicious. All serum samples with S/CO values > 0.9 were subjected to confirmatory testing via TPPA (Fujirebio, Tokyo, Japan). Specimens with positive TPPA results were considered positive. All experiments were performed according to the manufacturer's instructions.

Ethical considerations

This study was approved by the Ethics Committee of the First Affiliated Hospital of Soochow University (Ethical Number: 2023200). All participants provided written informed consent. In this study, only the number of positive cases, sex, and age were counted. All personal information was kept confidential.

Statistical analysis

Data were visualized using Graphpad Prism 7.0 software (La Jolla, CA, USA) and analyzed using the SPSS software version 20.0 (IBM Corp., Armonk, NY, USA). Descriptive analysis was used to calculate proportions and frequencies for all categorical variables. The ROC curve was

Table 1. Sex and age distribution of syphilis positive cases

Age (year)	Screening cases	Positive cases (S/CO ≥ 1)			Detection rate (%)	Men-to-women ratio	Chi-square test	Z-test
		Total	Men	Women				
0-18	4734	21	9	12	0.44	0.75	χ^2=229.508, P < 0.0001	φ=0.211, P < 0.0001
19-40	66832	778	276	502	1.16	0.55		
41-65	107128	2537	1486	1051	2.37	1.41		
> 65	68807	1837	1236	601	2.67	2.06		
Total	247501	5173	3007	2166	2.09	1.39		

Table 2. Comparison of the result of anti-T. pallidum antibodies using CLIA and TPPA

S/CO value	n	TPPA		Coincidence rate (%)	Chi-square test
		Negative	Positive		
0.90-0.99	157	133	24	15.29	χ^2=2059.16, P < 0.0001
1.00-5.00	1856	884	972	52.37	
5.01-15.00	1707	36	1671	97.89	
> 15.00	1610	0	1610	100.00	
Total	5330	1053	4277	80.24	

used to evaluate the diagnostic performance and the Youden index was used to calculate the optimal cut-off value. Statistically significant differences between groups were evaluated using the Chi-square test (Bonferroni method) or Fisher's exact test for categorical variables, and the Z-test for pairwise comparisons. Statistical significance was set at P < 0.05.

Results

Sex and age distribution

Of the 247,501 individuals screened between January 2019 and August 2021, 5,173 were found positive for syphilis (S/CO value ≥ 1) using the CLIA method, with a detection rate of 2.09% (**Table 1**). These cases were further subdivided by sex and age, and a Chi-square test (Bonferroni method) showed that these two parameters had an impact on the detection rate (χ^2=229.51, P < 0.0001). Pairwise comparison analysis revealed that there were differences in the detection rates for different age groups within the same sex; however, the detection rate was not significantly different for patients < 18 years of age (φ=0.21, P < 0.0001). Z-test analysis showed that sex-based factors only affected the detection rate of syphilis in younger (19-40 years old) and older individuals (> 65 years old). The former group was dominated by women, whereas the latter predominantly consisted men. These findings suggest that the focus of syphilis prevention and education varies according to sex and age.

Correlation between S/CO values and TPPA results

We performed TPPA on 5,330 samples that were initially screened as positive using CLIA, which gave us a total coincidence rate of 80.24% (**Table 2**). Statistical analysis showed that the coincidence rate of CLIA and TPPA increased with increases in the S/CO value (χ^2=2059.16, P < 0.0001). When the S/CO value was > 15, the coincidence rate of the two methods reached 100%, indicating a high probability of syphilis diagnosis. Notably, we set the S/CO value in the range of 0.9-0.99 as the gray zone, and conducted TPPA on the samples with S/CO values in this range. The results showed that 24 of the 133 samples were positive, with a PPV of 15.29%, suggesting that it is necessary to perform confirmatory tests on samples with S/CO values in the gray zone to prevent missed diagnosis.

Effects of different S/CO values on the detection of syphilis in different populations

To better analyze the relationship between the S/CO value and the syphilis detection rate, we divided the S/CO value into four intervals: the suspicious group (0.90-0.99), the low-value group (1.00-5.00), the median-value group (5.01-15.00), and the high-value group (> 15.00). As is shown in **Figure 1A**, positive cases were concentrated in the middle-aged (41-65 years) and older individuals (> 65 years) groups, and the detection rate increased with age. The proportion of cases detected in the low-value group (S/CO value: 1.0-5.0) was the highest (**Figure 1B**). In addition, most patients in the high-value group (S/CO value > 15.0)

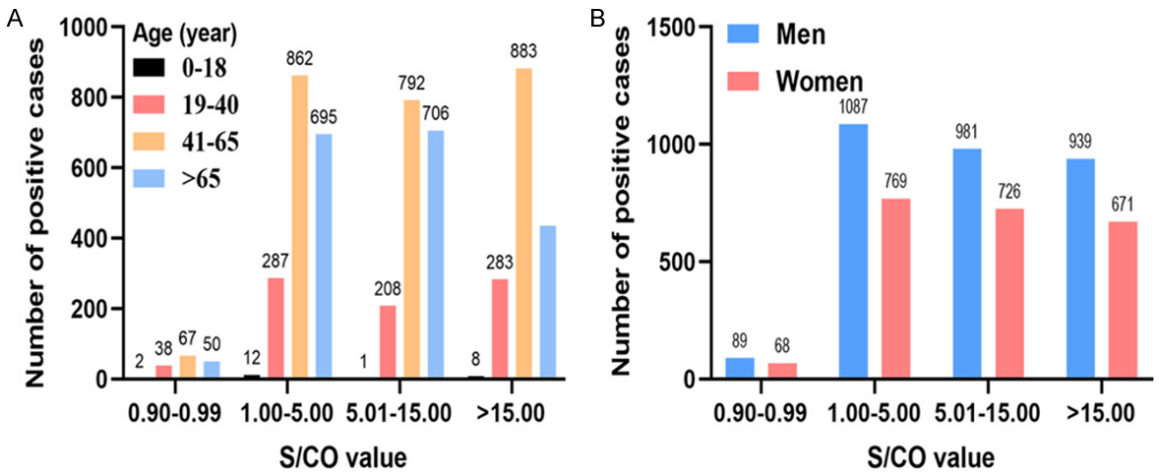

Figure 1. Effects of different S/CO values on syphilis detection. A. Relationship between different S/CO values and age of syphilis positive cases; B. Sex distribution of syphilis positive cases with different S/CO values.

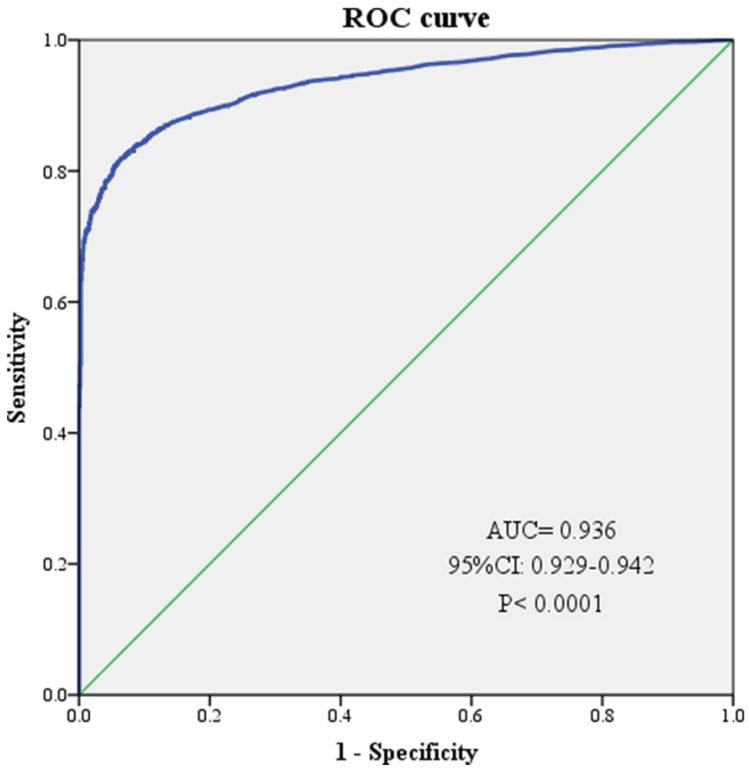

Figure 2. Use of ROC curve to find the suitable diagnostic threshold of CLIA.

were men aged 41-65 years, suggesting that more attention should be paid to the screening of these patients by relevant departments.

Use of receiver operator characteristic (ROC) curve to find the suitable diagnostic threshold of CLIA

The S/CO values of the 5,330 samples confirmed using TPPA were determined, confirming that 4,277 were positive and 1,053 were negative. The ROC curve showed that the use of the S/CO value for the diagnosis of syphilis had good diagnostic efficiency, with an area under curve (AUC) of 0.936 (95% confidence interval [CI]: 0.929-0.942, P < 0.0001; **Figure 2**). The Youden index was calculated using the formula: sensitivity + specificity - 1. The results showed that when the S/CO value was 3.945, the Youden index was the highest, suggesting that the S/CO value was the best diagnostic threshold for syphilis, with a sensitivity and specificity of 81.90% and 93.64%, respectively.

Annual and clinical department statistics on syphilis detection

The positive cases of syphilis that we analyzed involved most departments in the hospital, and the top six departments with the most positive cases were urology, orthopedics, cardiology, general surgery, gastroenterology, and gynecology (**Figure 3A**). From the perspective of year distribution, the syphilis detection rate in 2019-2021 was roughly the same (**Figure 3B**). Notably, the number of syphilis-positive cases dropped sharply in February 2020. This may be

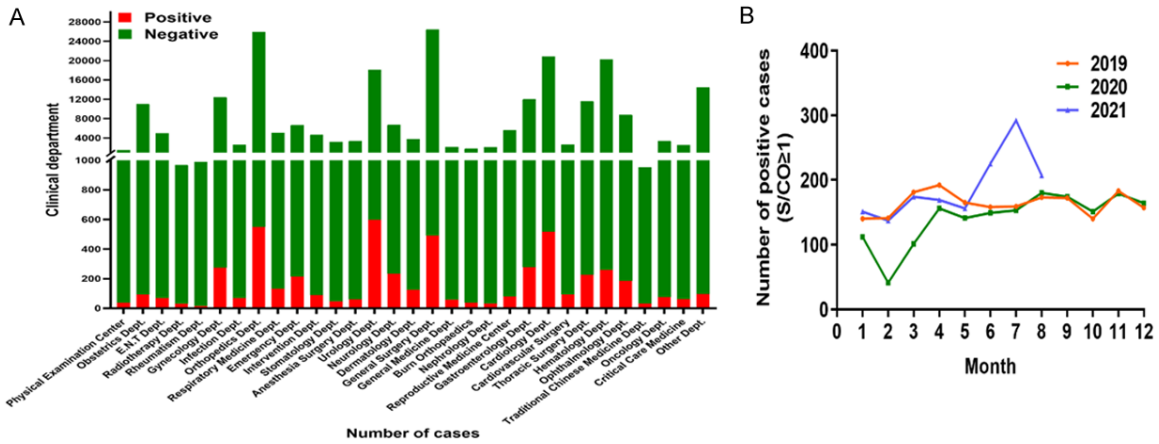

Figure 3. Annual and clinical department statistics of syphilis positive cases. A. Distribution of syphilis positive cases in different clinical departments; B. Monthly statistics of syphilis positive cases between 2019 and 2021.

related to quarantine measures advocated by the Chinese government in response to the spread of COVID-2019. However, the reason for the sudden increase in the syphilis detection rate in June and July 2021 is unclear and may warrant additional evaluation through further case collection.

Discussion

Syphilis is an STD that can affect multiple organ systems. Many regions in China have a remarkably high prevalence of syphilis, such as Beijing (23.57 cases per 100,000 in 2016) [12], Jiangsu (30.1 cases per 100,000 in 2015) [6], and Yunnan (0.51 cases per 100,000 in 2016) [13]. In our study, we screened 247,501 cases reported between January 2019 and August 2021, of which 5,137 positive cases were detected using CLIA, revealing a detection rate of 2.09%. This is approximately equivalent to that in Nanjing (2.01-2.07%) [14], the capital city of Jiangsu Province.

Our study revealed that the initial positive screening cases included 3,007 men and 2,166 women. Among them, middle-aged and older men > 41 years of age accounted for the vast majority, which may be related to their strong sexual intentions and men-to-men behavior. There seems to be an upward trend in syphilis infection among men who have sex with men (MSM) in China, with an infection rate of 7.1% in 2013-2015, and 8.3% in Jiangsu in 2015 [15, 16]. Therefore, men-to-men behavior is also an important factor that affects the prevalence of syphilis, and scientific education on sexual knowledge among key populations is necessary.

In China, the number of reported syphilis cases is increasing every year - particularly that of latent syphilis, which has increased dramatically from 14.2% to 73.6% [17]. The increase in the proportion of latent syphilis diagnoses is likely a result of the scale-up of syphilis screening, such as presurgical screening and co-screening with HIV, which is also conducive to the early detection and treatment of syphilis. Serological screening is the main method used to confirm suspected cases of syphilis infection. However, factors such as reagent lot numbers, manufacturers, and testing instruments cause variability in the sensitivity and specificity of detection reagents [18].

TPPA is recommended if there are discordant results between immunoassays and reflex non-treponemal testing [7, 9, 19]. In this study, 5,330 samples with S/CO values > 0.9 were verified using TPPA, of which 4,277 were double positive. With an increase in the S/CO value, the coincidence rates of CLIA and TPPA also increased, and a 100% coincidence rate was obtained when the S/CO was > 15. In addition, there were 24 TPPA-positive cases with S/CO values in the gray zone; therefore, samples with S/CO values of < 1 should be carefully re-examined to avoid missed diagnoses. As the PPV of the low-S/CO group was only 52.37%, the ROC curve was used to find the optimal diagnostic threshold. The results showed that an S/CO value of 3.945 was the

best diagnostic value for the CLIA method, with a diagnostic specificity of 93.64% and a sensitivity of 81.90%.

Syphilis can be divided into primary, secondary, recessive, neurosyphilis, and fetal syphilis. Primary syphilis usually manifests as a painless ulcer (chancre) that spontaneously resolves at the site of inoculation, a widespread maculopapular rash, and nonspecific systemic symptoms that characterize the secondary stage [9]. Symptoms of tertiary syphilis can involve many organs, including the brain (neurosyphilis), skin, bone (gumma), and cardiovascular system [20]. Our data showed that patients who were positive for syphilis were distributed across almost all hospital departments, and that the six departments with the most positive cases were urology, orthopedics, cardiology, general surgery, gastroenterology, and gynecology. The high number of positive patients in the urology, cardiology, and gynecology departments may be related to the common clinical manifestations of syphilis in the latent period, such as genital chancre, central nervous system, and cardiovascular system lesions. The large number of positive cases in the orthopedics and general surgery departments may be due to the large patient base and extensive screening of infectious disease pathogens. Further analysis showed that the middle-aged group (41-65 years old) was the main body of positive cases in the above departments, possibly because this group still retained a desire for sexual behavior, which in turn increased the transmission of syphilis. Wu et al. [21] found that the prevalence of syphilis increased significantly with age. However, in our study, adults > 65 years of age had a lower reported positivity rate than those in the 41-65 years age group, possibly because sexual shame and fear of age discrimination in older adults make them less likely to seek medical help.

Our study had several key limitations worth noting. First, this study only collected the screening information of patients who were positive for syphilis in a first-class tertiary hospital in Suzhou between 2019 and 2021, so the scope of case collection was limited both temporally and spatially. Second, owing to the urban silence policy during the COVID-19 pandemic, the mobility of the population decreased, leading to an underestimation of the actual positive detection rate of syphilis. In the future, we expect to continue to expand the sample size, extend the time span, and, where permitted, collaborate with other hospitals in the Suzhou area or the local authorities, such as Centers for Disease Control and Prevention (CDC), to refine our study. We also hope to link syphilis screening with other blood test indicators to provide a new auxiliary method for syphilis screening.

The prevalence of syphilis presents different characteristics in individuals of different sexes and ages. Medical institutions should carry out active detection and expanded screening for syphilis, particularly in older individuals and pregnant women. When performing serological screening, two or more methods should be used to avoid misdiagnosis and missed diagnosis. In addition, a comprehensive judgment should be made using both clinical symptoms and epidemiological history.

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Disclosure of conflict of interest

None.

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