Original Article

Characteristics of Patients with Primary and Late Latent Syphilis Patients Who Were Initially Non-Reactive to the Rapid Plasma Reagin Test

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SUMMARY: We aimed at determining the characteristics of patients with primary and late latent syphilis who were non-reactive on initial screening by rapid plasma reagin (RPR) but reactive by treponemal tests. We collected the RPR test results of all primary and late latent syphilis patients in our hospital from December 2000 to March 2012. The characteristics of syphilis patients who were non-reactive by RPR testing were compared to those of reactive patients. Multiple logistic regression was used to identify factors associated with non-reactive RPR results. Among primary syphilis patients, 37 (16.5%) were non-reactive on initial RPR and were compared with the 187 reactive cases. Age > 35 years was an independent factor associated with a non-reactive result in primary cases (odds ratio [OR], 95% confidence intervals [CI] = 3.55 [1.39–9.07]). Of the late latent patients, 61 (8.8%) were non-reactive by RPR and 636 were reactive. Age > 34 years was also an independent factor associated with a non-reactive result in late latent cases (OR [95% CI] = 4.30 [2.28–8.12]). This study suggests that RPR testing alone is insufficient to diagnose primary and late latent infections, especially in middle-aged and elderly individuals. Syphilis detection was lower for patients with primary syphilis than for those with late latent syphilis based on the results of the RPR.

INTRODUCTION

Syphilis is a spirochetal infection with multiple manifestations. In recent years, syphilis has reemerged as a significant public health problem in many countries, including China. Nationwide surveillance data indicate that the incidence rate of syphilis in China in 1993 was 0.2 per 100,000 persons, whereas that for primary and secondary syphilis was 5.7 per 100,000 persons in 2005 (1). According to nationwide surveillance data, there were 327,433 new cases of syphilis in China in 2009 (24.7 per 100,000 persons).

Despite the challenges in the interpretation of serological test results, non-treponemal tests are the most widely used for the diagnosis of syphilis. Non-treponemal tests include the rapid plasma reagin (RPR), venereal disease research laboratory (VDRL), and toluidine red unheated serum test. In China, RPR is the predominant test used for such screening. The RPR test is relatively inexpensive, widely available, and is useful for determining the efficacy of treatment by monitoring the antibody titer levels. However, RPR test sensitivity and specificity vary by stage, and its results, therefore, must be interpreted considering the stage of syphilis (2,3). It has long been recognized that the RPR test has poor

sensitivity in the late (4) and very early stages of syphilis. The test becomes reactive within 1–4 weeks after the initial infection. Approximately 30% of patients with early syphilis show non-reactive results in their initial RPR test (5). Other estimates indicate that approximately 15 % of patients with primary syphilis are seronegative at initial presentation, supporting the need for repeat testing after 2–4 weeks, and confirmatory testing (6). In our study, we aimed to identify characteristics of syphilis patients in Beijing, China, who were initially non-reactive on RPR testing.

METHODS

Study population: Demographic, clinical, and laboratory data of 921 patients with primary and late latent syphilis were retrospectively analyzed. All of them were outpatients who visited the sexually transmitted disease (STD) and infectious disease clinic of Peking Union Medical College Hospital, Beijing, China, from December 2000 to March 2012. According to national guidelines (7), primary syphilis was defined as a clinically compatible case characterized by the presence of ≥ 1 chancre and inguinal lymphadenopathy, laboratory confirmation of *Treponema pallidum* in clinical specimens by RPR and treponemal *T. pallidum* hemagglutination (TPHA), or particle agglutination assay for antibody to *T. pallidum* (TPPA) and/or fluorescent treponemal antibody absorption (FTA-Abs). Latent syphilis was defined as an asymptomatic case with a possible history of infection supported by reactive RPR, reactive treponemal test, and normal cerebrospinal fluid. When initial infection has occurred more than 12

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months prior, latent syphilis is classified as late. Patients were excluded if they were HIV-positive, had neurosyphilis, or had a prior diagnosis of syphilis or if the initial serology occurred prior to the study period.

Laboratory testing: Blood (4 ml) was collected from each patient during their first visit to our STD and infectious clinic. The blood samples were subsequently transported to the laboratory where trained laboratory personnel performed RPR, TPPA, and/or FTA-Abs (Quality control: Syphilis Serology Proficiency Testing Survey, Laboratory Code No. 990077; Centers for Disease Control and Prevention, Atlanta, Ga., USA and World Health Organization, Geneva, Switzerland). Each test was performed in accordance to manufacturer's instructions. As a routine in our STD clinic, after treatment, all the patients were asked to periodically review their clinical symptoms and serum RPR titers every 3 months. Patients with early syphilis who were initially found to be non-reactive on initial RPR testing were asked to take a retest 2–4 weeks later. Only cases with RPR sero-converted were included in our study, and patients with two consecutive negative RPR tests were excluded. The patient's stage of syphilis was assessed based on physical examination and serology results using previously described criteria (7).

Statistical analysis: For the purpose of this analysis, demographic, clinical, and laboratory data were collected for all cases of primary and late latent syphilis for the study period. Bivariate and multivariate regression models were developed to examine odds ratios (ORs) with 95% confidence intervals (CI) for characteristics associated with non-reactive RPR tests. Variables with a significance level of $P < 0.05$ in the bivariate analysis were retained in the multivariate regression model.

RESULTS

There were 224 cases of primary syphilis (53 women and 171 men; mean age, 38 years; median age, 37 years [15–80 years]). Majority of patients were of Han nationality (Table 1). Most (94.0%) of the patients were heterosexual. Among the 224 primary syphilis cases, 37 (16.5%) were non-reactive on initial RPR screening and converted to RPR positive 1–2 months after the onset of chancres and/or inguinal lymphadenopathy. In unadjusted bivariate analyses (Table 2), primary syphilis with a non-reactive initial RPR was more likely to occur among patients older than 35 years of age and patients without coinfection with other STDs. There was no statistically significant association found with gender, ethnicity, residence, occupation, education, sexual practice, condom use, or number of sex partners. Multivariate analyses showed that age > 35 years was an independent predictor of non-reactive RPR test result (OR [95% CI] = 3.55 [1.39–9.07]).

There were 697 cases of late latent syphilis (317 women and 380 men; mean age, 37.7 years; median age, 34 years [16–87 years]). The majority patients were of Han nationality (Table 1). Most (97.8%) of the patients were heterosexual. Among the 697 patients with late latent syphilis, 61 (8.8%) were found to be non-reactive on initial RPR screening (patients who were non-reactive on initial RPR in other hospitals but were reactive in our clinic). In unadjusted bivariate analyses (Table 3), late

Table 1. Distribution of patient characteristics by the stage of syphilis

Characteristic	Primary (<i>n</i> = 224)	Late latent (<i>n</i> = 697)
	No. (%) ¹⁾	No. (%) ¹⁾
Age (yr)		
Mean [SD]	38.0 [12.6]	37.7 [13.8]
Median [range]	37 [15–80]	34 [16–87]
15–24	32 (14.3)	85 (12.2)
25–34	64 (28.6)	276 (39.6)
35–44	63 (28.1)	156 (22.4)
45–54	39 (17.4)	100 (14.3)
≥ 55	26 (11.6)	80 (11.5)
Gender		
Male	171 (76.3)	380 (54.5)
Female	53 (23.7)	317 (45.5)
Ethnicity		
Han	94 (89.5)	457 (96.6)
Minority	11 (10.5)	16 (3.4)
Residence		
Rural areas	86 (39.1)	327 (47.1)
Urban areas	134 (60.9)	367 (52.9)
Occupation		
Worker	41 (19.9)	96 (17.0)
Unemployed	38 (18.4)	68 (12.0)
Self-employed	37 (18.0)	94 (16.6)
Farmer	26 (12.6)	92 (16.3)
Government employee	25 (12.1)	59 (10.4)
Merchant	17 (8.3)	62 (11.0)
Student	14 (6.8)	50 (8.8)
Others	8 (3.9)	44 (7.8)
Education		
No-education	7 (3.7)	13 (2.8)
Primary	51 (27.0)	128 (27.4)
Secondary	104 (55.0)	239 (51.1)
Higher	27 (14.3)	88 (18.8)
Sexual practice		
Heterosexual	126 (94.0)	585 (97.8)
Homo/bisexual	8 (6.0)	13 (2.2)
Condom use		
Consistent use	34 (29.3)	139 (32.6)
Inconsistent or never use	82 (70.7)	288 (67.4)
No. of sex partners		
0–1	50 (61.0)	269 (62.1)
2–3	17 (20.7)	109 (25.2)
4–5	10 (12.2)	35 (8.1)
> 5	5 (6.1)	20 (4.6)
Coinfection with other STDs		
Yes	21 (15.7)	58 (11.3)
No	113 (84.3)	457 (88.7)

¹⁾: Percentage within columns among subjects with non-missing data.

SD, standard deviation; STD, sexually transmitted-disease.

latent syphilis with a non-reactive initial RPR was more likely to occur among patients with age > 34 years and patients living in urban areas. There was a marginal association between the presence of syphilis and having more than 5 sex partners (OR [95% CI] = 3.78 [0.97–14.68]). We found no significant association with gender, ethnicity, occupation, education, sexual practice, condom use, number of sex partners, or coinfection.

Table 2. Characteristics associated with non-reactive RPR among primary syphilis patients

Characteristic	Non-reactive case	Reactive case	%	OR (95% CI)	AOR (95% CI)
Age (yr)					
≤35	8	94	7.8	1	1
>35	29	93	23.8	3.66 (1.59–8.43)	3.55 (1.39–9.07)
Gender					
Female	7	46	13.2	1	—
Male	30	141	17.5	1.40 (0.58–3.4)	—
Ethnicity					
Han	22	72	23.4	1	—
Minority	3	8	27.3	1.23 (0.3–5.03)	—
Residence					
Rural areas	13	73	15.1	1	—
Urban areas	22	112	16.4	1.10 (0.52–2.32)	—
Occupation					
Worker	6	35	14.6	1	—
Unemployed	7	31	18.4	1.32 (0.40–4.34)	—
Self-employed	6	31	16.2	1.13 (0.33–3.87)	—
Farmer	4	22	15.4	1.06 (0.27–4.19)	—
Government employee	4	21	16.0	1.11 (0.28–4.40)	—
Merchant	3	14	17.6	1.25 (0.27–5.71)	—
Student	3	11	21.4	1.59 (0.34–7.44)	—
Others	1	7	12.5	0.83 (0.09–8.04)	—
Education					
No education	1	6	14.3	1	—
Primary	16	35	31.4	2.74 (0.31–24.71)	—
Secondary	15	89	14.4	1.01 (0.11–9.00)	—
Higher	5	22	18.5	1.36 (0.13–14.00)	—
Sexual practice					
Heterosexual	30	96	23.8	1	—
Homo/bisexual	1	7	12.5	0.46 (0.05–3.87)	—
Condom use					
Consistent use	5	29	14.7	1	—
Inconsistent or never use	24	58	29.3	2.4 (0.83–6.94)	—
No. of sex partners					
0–1	10	40	20.0	1	—
2–3	4	13	23.5	1.23 (0.33–4.60)	—
4–5	2	8	20.0	1.00 (0.18–5.46)	—
>5	1	4	20.0	1.00 (0.10–9.96)	—
Coinfection with other STDs					
Yes	1	20	4.8	1	1
No	32	81	28.3	7.90 (1.02–61.35)	5.32 (0.66–42.80)

OR, odds ratio; AOR, adjusted odds ratio; CI, confidence interval.

tion with other STDs. In multivariate analyses, a non-reactive RPR result was associated with an age >34 years (OR [95% CI] = 4.30 [2.28–8.12]) and marginally associated with urban residence (OR [95% CI] = 1.75 [0.99–3.08]).

DISCUSSION

Because of the difficulties associated with implementing the World Health Organization's recommended combination of non-treponemal and treponemal serological tests for screening of syphilis cases (8), many areas in China only screen using non-treponemal tests, usually the RPR. However, RPR reactivity may show false-negative or non-reactive results because of prozone phenomenon (9), test misinterpretation, or laboratory error in performing the test (3). Undetected syphilis can lead to ongoing transmission of infection and can

allow an existing infection to progress to more severe stages of syphilis. Given the public health consequences of a false-negative screening result, we identified characteristics of patients in Beijing, China, who were initially non-reactive on RPR testing.

To our knowledge, this is the first study from Asia to characterize primary and late latent syphilis patients who were initially non-reactive on RPR testing. As a retrospective study, the analysis was restricted to routinely collected medical data. Nevertheless, the findings highlight important factors that may be useful to consider when screening with an RPR. In our study, patients with age >35 years were 3–4 times more likely to be non-reactive on RPR testing in both primary and late latent syphilis. One hypothesis we proposed to explain the phenomenon is lack of immunity. As we know, cell-mediated immune response is important for protection against *T. pallidum*. In primary cases, im-

Table 3. Characteristics associated with non-reactive RPR among late latent syphilis patients

Characteristic	Non-reactive case	Reactive case	%	OR (95% CI)	AOR (95% CI)
Age (yr)					
≤34	13	348	3.6	1	1
>34	48	288	14.3	4.46 (2.37–8.40)	4.30 (2.28–8.12)
Gender					
Female	28	289	8.7	1	—
Male	33	347	8.8	1.02 (0.60–1.73)	—
Ethnicity					
Han	11	446	2.4	1	—
Minority	1	15	6.3	2.70 (0.33–22.31)	—
Residence					
Rural areas	20	307	6.1	1	1
Urban areas	41	326	11.2	1.93 (1.11–3.37)	1.75 (0.99–3.08)
Occupation					
Worker	5	91	5.2	1	—
Unemployed	8	60	11.8	2.43 (0.76–7.77)	—
Self-employed	9	85	9.6	1.93 (0.62–5.98)	—
Farmer	7	85	7.6	1.50 (0.46–4.90)	—
Government employee	8	51	13.6	2.86 (0.89–9.19)	—
Merchant	7	55	11.3	2.32 (0.70–7.66)	—
Student	6	44	12.0	2.48 (0.72–8.58)	—
Others	3	41	6.8	1.33 (0.30–5.84)	—
Education					
No education	2	11	15.4	1	—
Primary	13	115	10.2	0.62 (0.12–3.12)	—
Secondary	25	214	10.5	0.64 (0.14–3.07)	—
Higher	9	79	10.2	0.63 (0.12–3.29)	—
Sexual practice					
Heterosexual	43	542	7.4	1	—
Homo/bisexual	1	12	7.7	1.05 (0.13–8.27)	—
Condom use					
Consistent use	3	136	2.2	1	—
Inconsistent or never use	8	280	2.8	1.30 (0.34–4.96)	—
No. of sex partners					
0–1	12	257	4.5	1	—
2–3	4	105	3.7	0.82 (0.26–2.59)	—
4–5	2	33	5.7	1.30 (0.28–6.06)	—
>5	3	17	15.0	3.78 (0.97–14.68)	—
Coinfection with other STDs					
Yes	12	445	2.6	1	—
No	3	55	5.2	2.02 (0.55–7.39)	—

Abbreviations are in Table 2.

munity is lower in elderly people; therefore, they cannot produce antibodies as fast as younger people. In late latent cases, because of the lower immunity, *T. pallidum* cannot be cleared completely. The remaining *T. pallidum* can stimulate the body to produce low concentration antibodies, which is difficult to detect, and false-negative results for RPR testing may occur more easily, leading to test misinterpretation or laboratory error in performing the test in those patients. Unlike a similar study in Canada by Singh et al. (10), in which men were more likely than women to be non-reactive on RPR testing for both primary and late latent syphilis. Caucasians were more likely than other ethnic groups to be non-reactive with RPR among primary syphilis cases. However, aboriginal and African-American patients were more likely to be non-reactive in late latent syphilis. Patients diagnosed during 1980–1986 were more likely to be non-reactive with RPR among primary and

latent syphilis. Our study did not reveal any statistically significant association of the presence of syphilis with gender. Given that RPR testing is the screening method for syphilis in most parts of China, the results of this study suggest that patients older than 35 years should be more closely monitored because they might be prone to show non-reactive results when screened with this test. Our data also support the recommendation that all patients suspected with primary and late latent syphilis should be screened at baseline with both non-treponemal and treponemal tests or with only treponemal tests. Patients with early syphilis who are initially found to be non-reactive by a non-treponemal test should be retested 2–4 weeks later. Moreover, careful observation of primary syphilis continues to be warranted in certain situations, such as those in which epidemiological exposure to syphilis is suspected and/or when relevant clinical findings are present.

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Conflict of interest None to declare.

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