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MAJOR ARTICLE

Changes in the Syphilis Rapid Plasma Reagin Titer Between Diagnosis and Treatment

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Background. We compared the rapid plasma reagin (RPR) titer on the day of initial presentation with that on the day of syphilis treatment to inform clinical practice as to whether a repeated RPR test should be recommended.

Methods. We undertook a retrospective study between 1 March 2011 and 31 December 2020 at the Melbourne Sexual Health Centre in Australia among individuals who underwent syphilis serology on the day of initial presentation and the day of treatment, if the latter were within 14 days after initial presentation. We calculated the percentage of individuals with a ≥4-fold change in RPR titer, stratified by the time between initial presentation and treatment and by syphilis stage.

Results. Among the 766 included syphilis cases, the median duration between initial presentation and treatment was 6 days (interquartile range, 5–7 days). Of these cases, 14.8% (n = 113) had a ≥4-fold increase or decrease during this interval. The number of cases with a ≥4-fold increase or decrease in RPR titer increased with increasing time between initial presentation and treatment, from 5.7% (n = 6) 1–3 days after initial presentation to 26.2% (n = 27) at 10–14 days ($P_{trend} < .001$). There was no significant difference in the number of cases with a ≥4-fold increase or decrease in RPR titer between syphilis stages ($P = .66$).

Conclusions. Our data support the recommendation of repeating the RPR titer if the day of initial presentation and the day of treatment are different, even when treatment is within a few days after initial presentation.

Keywords. rapid plasma reagin; treatment; diagnosis; sexually transmitted infection; sexually transmitted disease.

The incidence of syphilis has been rising globally over the last 2 decades [1–3]. In Australia, the number of new cases of infectious syphilis has increased by 206%, from 7.8 cases per 100 000 in 2013 to 23.9 per 100 000 in 2019 [4], and cases disproportionally affect gay, bisexual, and other men who have sex with men and Aboriginal and Torres Strait Islanders [5]. Since the late 2010s, the syphilis epidemic has become more generalized in Australia, with substantial increases in heterosexuals [6]. The increased incidence of syphilis has renewed interest in the recommendations for testing and treatment. One of these recommendations is to perform a rapid plasma reagin (RPR) titer test on or close to the day of treatment [7–9]. The RPR test is usually performed when a treponemal test result is positive and can be used to monitor response to treatment [10, 11]. However, to our best knowledge, no published data have been provided in the US, UK, or Australian guidelines to support per-treatment RPR testing [7–9]. This recommendation is important because if the RPR titer rises between the day of initial presentation and the day of treatment and the test is not repeated, the baseline titer against which treatment response is monitored would be incorrect.

Successful treatment of primary and secondary syphilis is conventionally defined as a ≥4-fold reduction in RPR titer within 12 months [7–9]. The guidelines from the US Centers for Disease Control and Prevention define successful treatment in patients with latent syphilis as a ≥4-fold reduction in RPR titer within 24 months [9]. Additional considerations are required for patients with complicated syphilis, neurosyphilis, tertiary syphilis, or coinfection with human immunodeficiency virus (HIV) infection [7–9]. Furthermore, it may result in further unnecessary investigations or treatment if the initial treatment was incorrectly deemed to have failed by not capturing the highest titer.

This study aimed to determine the changes in RPR titer between the day of initial presentation and the day of treatment and whether increases in the titer during this period are sufficient to recommend that the titer is repeated at the time of treatment. We used retrospective data from a large Australian sexual health service to determine changes in the RPR titer

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between the time of initial presentation and the day of receiving treatment.

METHODS

Study Setting and Population

We conducted a retrospective cohort study using electronic patient data collected at the Melbourne Sexual Health Centre (MSHC) between March 2011 and December 2020. The MSHC is a publicly funded HIV and sexually transmitted infection clinic in Melbourne, Australia, which provides free HIV and sexually transmitted infection testing and treatment.

We included individuals who were aged ≥16 years, had an initial presentation of syphilis between 2011 and 2020, had an RPR titer of ≥1 at the time of initial presentation, returned to the MSHC for treatment within 14 days after the diagnostic RPR result on the day of initial presentation, did not receive treatment for syphilis on the day of initial presentation, and had titers obtained on both the day of initial presentation and the day of treatment. As part of routine clinical practice, all syphilis diagnoses are reviewed and staged around the time of diagnosis by an experienced sexual health clinician based on clinical information and laboratory results.

Syphilis Serology

Serology testing for syphilis included an RPR test (Becton Dickinson), a *Treponema pallidum* particle agglutination assay (Fujirebio), and either a *T. pallidum* enzyme-linked immunosorbent assay (Trepanostika EIA; BioMerieux), before January 2016, or a chemiluminescent immunoassay (LIAISON *Treponema* screen; DiaSorin), after January 2016. All laboratory tests were performed at the Victorian Infectious Diseases Reference Laboratory. Serology results were usually available within 24–48 hours after sample collection.

Statistical Analysis

Individuals who met the study criteria were reported along with their syphilis stage, RPR titer at treatment and initial presentation, and treatment type. We calculated the median with interquartile range (IQR) and mean with standard deviation of days between initial presentation and treatment day RPR titers for each calendar year.

We calculated the median difference in RPR titers between the day of initial presentation and the day of treatment. Given that the titers were measured using the double dilution method, the data were logarithmically (base 2) transformed when we calculated the difference between 2 titers. A ≥4-fold difference in RPR titer was defined as a $\log_2$ fold-change of ≥2. Samples were grouped by the number of days between initial presentation and treatment, and a χ^2 test for trend was used to determine the changes in the proportion of cases with a ≥4-fold difference in RPR titer with increasing number of days between initial presentation and treatment. Cases were stratified into 3 categories based on infectious syphilis stage: primary, secondary, or early latent. Early latent syphilis was defined as asymptomatic syphilis occurring within the last 2 years [12]. We were unable to stage all cases owing to the lack of information. We performed χ^2 tests to compare the proportions of cases with a ≥4-fold increase or decrease in RPR titer (≥4 fold) by syphilis stage and the differences in these proportions between those with HIV and those without HIV.

We performed univariable logistic regression to examine the association between cases with a ≥4-fold increase or decrease in RPR titer (dependent variable) and the titer at initial presentation (covariate). We also performed multivariable logistic regression by adjusting the syphilis stage and the number of days between initial presentation and treatment. Crude and adjusted odds ratios (ORs) and the corresponding 95% confidence intervals (CI) were presented. The RPR titer may also increase after treatment [13]. In cases with a ≥4-fold increase in RPR titer, we also examined the titer after treatment. Analyses were performed using Epi Info 7 and Stata (version 17) software. This study was approved by the Alfred Hospital Ethics Committee, Melbourne, Australia (project no. 805/20).

RESULTS

Between March 2011 and December 2020, a total of 165 929 individuals attended MSHC at least once. Of these, syphilis serology was performed at least once in 96 158 (58.0%). A total of 4903 positive serology results were recorded. The number of new positive syphilis serology results increased nearly 3-fold from 118 in 2011 to 370 in 2020 [14]. We excluded 4136 of 4903 cases (84.4%) in which individuals had testing on the same day as their treatment or did not have any subsequent syphilis serology performed within 14 days after the initial presentation. The remaining 766 cases (121 primary, 100 secondary, 339 early latent, 31 late latent, and 175 unclassified) were included in this analysis; 95.6% of patients (n = 732) were men, 3.0% (n = 23) were women, and 1.4% (n = 11) were gender diverse (Supplementary Table 1). Of the 766 cases, 15.1% (n = 116) were repeated infections from the same individuals. The median age (IQR) was 33 (27–43) years, and the median time (IQR) between initial presentation and treatment was 6 (5–7) days.

Of the cases, 14.8% (113 of 776) had a ≥4-fold increase (83.2% [94 of 113]) or decrease (16.8% [19 of 113]) in RPR titer between the day of initial presentation and the day of treatment (Table 1): 22 had primary syphilis, 19 had secondary syphilis, 53 had early latent syphilis, and 19 were unclassified. The median RPR titer (IQR) was 1:32 (1:12 to 1:192) at initial presentation and 1:32 (1:8 to 1:128) on the day of treatment. There was a 1-fold increase in the median titer between the day of initial presentation and the day of treatment for primary and secondary cases but no changes for early latent cases (Table 1). There

Table 1. Rapid Plasma Reagin Titer Findings in Syphilis Cases Stratified by Syphilis Stage

Finding	All Stages (N = 766)	Primary Stage (n = 121)	Secondary Stage (n = 100)	Early Latent Stage (n = 339)	Late Latent or Unclassified stage (n = 206)[a]
Time between initial presentation and treatment, median (IQR), d	6 (5–7)	6 (5–7)	6 (5–7)	6 (5–8)	6 (5–8)
RPR titer at initial presentation, median (IQR)	1:32	1:16	1:64	1:32	1:16
RPR titer at treatment, median (IQR)	1:32	1:32	1:128	1:32	1:16
Change in median RPR titer between initial presentation and treatment	No change	1-Fold increase	1-Fold increase	No change	No change
Cases with ≥4-fold increase or decrease in RPR titer, no. (%)[b]					
≥4-Fold increase or decrease	113 (14.8)	22 (18.2)	19 (19.0)	53 (15.6)	19 (9.2)
≥4-Fold increase	94 (12.3)	19 (15.7)	17 (17.0)	45 (13.3)	13 (6.3)
≥4-Fold decrease	19 (2.5)	3 (2.5)	2 (2.0)	8 (2.4)	6 (2.9)

Abbreviations: IQR, interquartile range; RPR, rapid plasma reagin.

[a]These 206 cases included 31 late latent syphilis cases (>2 years); 175 cases could not be staged owing to lack of information.

[b]There was no significant difference in the proportions of cases with a ≥4-fold increase or decrease in RPR titer across the 3 syphilis stages (primary, secondary and early latent) excluding late latent or unclassified stage ($P = .66$).

was no significant difference in the proportions of cases with a ≥4-fold increase or decrease in RPR titer across all 3 syphilis stages ($P = .37$). The number of cases with a ≥4-fold increase or decrease increased significantly with increasing number of days between initial presentation and treatment (Figure 1 and Table 2), from 5.7% (6 of 106) within 1–3 days to 26.2% (27 of 103) 10–14 days after initial presentation ($P_{trend} < .001$). There was no significant association between initial RPR titer and a ≥4-fold increase or decrease (OR, 1.00 [95% CI, 1.00–1.00]; $P = .22$), and the nonsignificant association remained the same in the multivariable analysis after adjustment for syphilis stage and the number of days between 2 titers (adjusted OR, 1.00 [1.00–1.00]; $P = .28$).

Thirty-five case patients were individuals with HIV infection. The RPR titer on the day of the initial presentation did not differ between those with HIV and those without HIV ($P = .13$). In 3 of these 35 cases, a ≥4-fold increase or decrease in titer was recorded. There was no difference in the proportions of cases with a ≥4-fold increase or decrease in RPR titer between those with HIV and those without HIV ($P = .29$).

We analyzed the data to determine the proportion of cases with a ≥4-fold increase in RPR titer between initial presentation and treatment that were falsely classified as serofast if the RPR on the day of initial presentation was the only titer available to assess whether the requisite 4-fold decrease had occurred. Of the 94 cases with a ≥4-fold increase in RPR titer, 59 had repeated RPR testing after treatment. The median duration after treatment for testing was 271 days. Five cases were incorrectly classified as not responding adequately to treatment if the RPR titer on the day of treatment was not used.

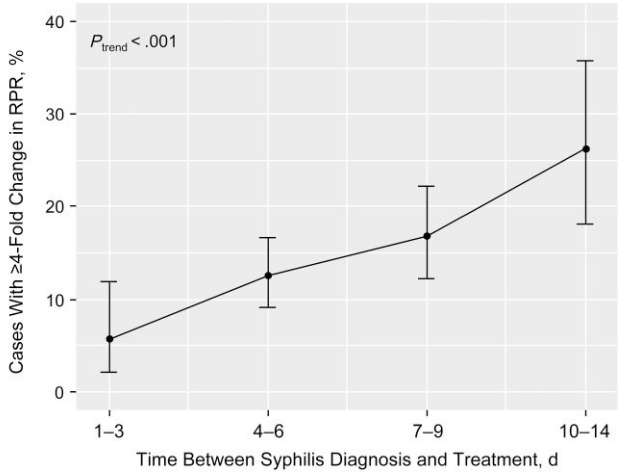

Figure 1. Proportion of syphilis cases with a ≥4-fold change in rapid plasma reagin (RPR) titer, stratified by the number of days between initial presentation and treatment. (P_{trend} was calculated using the χ^2 test for trend, and errors bars represent 95% confidence intervals.)

DISCUSSION

In the current study, we found that approximately 1 in 8 individuals with syphilis had an RPR titer that had increased or decreased by ≥4-fold between initial presentation and treatment.

Table 2. Syphilis Cases With a ≥4-Fold Increase or Decrease in Rapid Plasma Reagin Titer Between Initial Presentation and Treatment, Stratified by Syphilis Stage

Time Between Initial Presentation and Treatment	Cases With ≥4-Fold Increase or Decrease in RPR Titer, by Syphilis Stage, No./Total No. of Cases (%)[a]			
	All Stages (N = 766)	Primary (n = 121)	Secondary (n = 100)	Early Latent (n = 339)
1–3 d	6/106 (5.7)	1/25 (4.0)	1/14 (7.1)	3/40 (7.5)
4–6 d	40/319 (12.5)	6/44 (13.6)	12/46 (26.1)	16/148 (10.8)
7–9 d	40/238 (16.8)	16/44 (36.4)	4/31 (12.9)	18/100 (18.0)
10–14 d	27/103 (26.2)	2/8 (25.0)	2/9 (22.2)	16/51 (31.4)

Abbreviation: RPR, rapid plasma reagin.

[a]There were 31 late latent syphilis cases, and 175 syphilis cases could not be staged owing to lack of information. These 206 cases are included in the "All Stages" column but not otherwise displayed.

The proportion of cases with a ≥4-fold increase in titer rose significantly with increasing number of days between initial presentation and treatment, from 6% within 1–3 days after initial presentation to 27% within 10–14 days. Furthermore, 5 of 59 cases (8%) would have been incorrectly classified as serofast if RPR testing was not performed on the day of treatment.

The clinical significance of this finding is that a substantial proportion of individuals who are treated without a repeated RPR titer on the day of treatment may have their response to treatment misinterpreted because the median RPR titer on the day of initial presentation was lower than that on the day of treatment. If the titer had risen by ≥4-fold between initial presentation and treatment, then the case may incorrectly *appear* to have not responded satisfactorily to treatment, when in fact it had. This poses a risk of unnecessarily increased duration of treatment, unnecessary interventions, such as further investigation (including lumbar puncture) or repeated treatment, and potential associated harm from adverse effects.

These data are the first we are aware of concerning this issue, and they provide an evidence base for the recommendation that serology should be undertaken on the day of treatment to better guide treatment responses; however, doing so may be cost prohibitive in resource-limited settings and also has some logistic challenges in settings with undeveloped laboratory infrastructures.

Our study has several limitations. First, it used retrospective data collected from a single urban sexual health center that included mostly adult men, and therefore the results may not be generalizable to other populations and settings. Second, we included only cases in which initial presentation and treatment were ≤14 days apart, and a significant difference in RPR titers may be seen in cases where this interval is >14 days. Third, the number of cases in our study did not provide adequate power to determine with sufficient precision whether there was a significant difference in RPR titer changes between initial presentation and treatment according to syphilis stage, although we hypothesize that secondary syphilis would be more associated with rapid changes [15]. Fourth, we included only about 8% of cases treated for syphilis at our service because most individuals with syphilis were either treated immediately or treated elsewhere and were therefore not included. If these cases were systematically different from all syphilis cases our results may not be accurate, although we have no reason to consider this to be true. Finally, high RPR titers were recorded as either >1:512 or >1:1024 where the laboratory decided on the cutoff point to stop titrating, and the highest value was recorded for the analysis. This may underestimate the changes in the RPR titers of some individuals with a high titer at initial presentation.

Our study also had several strengths. First, it included a high number of analyzable cases that provided reasonable confidence intervals around our estimates. Second, we used the same laboratory for our assays over the entire period, and they undertake the RPR titer analyses in parallel, so these differences are likely less likely to reflect laboratory variation in the assay. Third, all cases were carefully reviewed for their stage by a senior sexual health clinician, so the clinical stages are likely to be correct.

In conclusion, to our knowledge, the current study is the first to investigate the necessity of a repeated RPR titer on the day of syphilis treatment. Our research can further be extended to investigate the necessity for a reduction in days between initial presentation and treatment as this may affect treatment outcomes and control of syphilis.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Author contributions. C. K. F. and E. P. F. C. conceived and designed the study. K. P. performed the data analysis and wrote the first draft of the manuscript. E. T. A. reviewed records of syphilis cases. E. P. F. C. provided statistical advice. All authors helped interpret data, revised the manuscript for intellectual content, and approved the final version of the manuscript, and E. P. F. C. had full access to all the data in the study and final responsibility for the decision to submit for publication.

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Potential conflicts of interest. M. Y. C. reports NHMRC Partnership Project Grant APP2003399. C. S. B. received NHMRC Leadership Grant 1173361 and is a board member for the International Society for STD Research and an unpaid consultant to the World Health Organization. J. J. O. reports investigator grants from the NHMRC and is the board director of the Australasian Society for HIV, Viral Hepatitis and Sexual Health Medicine, Australian Federation of AIDS Organisation and an Executive Committee member for the Royal Australian College of Physicians Chapter of Sexual Health Medicine. E. T. A. reports a Research Training Program Scholarship from Monash University and a Research Entry scholarship from the Chapter of Sexual Health Medicine, Royal Australasian College of Physicians. E. P. F. C. reports an NHMRC Emerging Leadership Grant (GNT1172873) and payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing, or educational events from CSL Seqirus and MSD, all paid to the institution. All other authors report no potential conflicts.

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