Syphilis treatment and HIV infection in a population-based study of persons at high-risk for STD/HIV infection in Lima, Peru

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Footnotes

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Short Summary
A study of individuals at high-risk for STDs in Peru found that syphilis was common, difficult to treat in those with low RPR titers, and highly associated with HIV infection.

Abstract
Objectives: Characterize syphilis epidemiology and the relationship of HIV status and initial RPR titer to syphilis treatment in Lima, Peru.
Study Design: We screened 1,261 individuals at high-risk for STDs for syphilis and HIV infection. Syphilis was treated with penicillin injection or doxycycline; treatment was extended in unresponsive cases.
Results: The prevalence of syphilis was 7.7%, one-year incidence 4.7%, and re-infection rate 42.7%. The treatment success rate was 93.4% (71/76); those with initial RPR titers ≤1:8 were less often treated successfully (86.8% vs. 100%, p=0.054) and required additional treatment more often (26.2% vs. 7.7%, p=0.028) than those ≥1:16. HIV infection was associated with syphilis, prevalent in 15.6% and 3.7% of those with and without syphilis, respectively (p<0.001), but did not affect treatment success (90.9% vs. 93.8%).
Conclusions: Syphilis was common, associated with HIV infection, and less responsive to therapy in those with initial RPR titers ≤1:8. HIV infection did not affect syphilis treatment success rates.

Key Words: Syphilis, HIV, Public Health, Peru, RPR

Introduction

Infection with other sexually transmitted diseases has been shown to increase the risk of HIV transmission.[1,2,3] This association is particularly apparent among ulcerative infections, such as herpes simplex virus type 2, chancroid, and syphilis, where HIV transmission is increased two to five times secondary to increased viral shedding and facilitated virus entry.[4,5] Improved STD management is efficacious in reducing HIV transmission in certain populations.[6]

Primary syphilis, the result of infection with the spirochete *Treponema pallidum*, typically results in a painless ulcer at the site of infection about three weeks after exposure.[7] If untreated, systemic spread leads to the development of secondary syphilis in approximately 25% of cases, which is associated with fever, lymphadenopathy, and diffuse rash. Symptoms often resolve spontaneously, despite continued presence and replication of the organism, with occasional relapses up to five years later.[8] Latent syphilis, the persistence of positive serology tests without symptoms, may be divided into early and late, with early-latent syphilis delineated as infection within the past year and late-latent as infection of greater than a year (or unknown) duration. Tertiary syphilis, with a lifetime incidence of about 25% among those not treated at an earlier stage, encompasses the major complications of untreated infection and is manifest most commonly as neurosyphilis, cardiovascular syphilis, or gummatous syphilis.[8] Syphilis infection in pregnant women carries the additional danger of congenital syphilis.[7]

The treatment of syphilis remains part science and part professional habit. Despite being the treatment of choice for decades, penicillin remains extremely active against *T. pallidum*. While not backed by major comparison studies on dosages or regimens, the Centers for Disease Control (CDC) recommends a single-dose, 2.4-million unit (MU) penicillin benzathine G intramuscular (IM) injection (or two weeks of a tetracycline) for early syphilis, and three consecutive weekly penicillin injections for late-latent and tertiary syphilis. Internationally, there does not exist a treatment consensus, as regimens and medications vary by

region.[9] Treatment is judged to be successful with at least a four-fold decline in nontreponemal antibody titer in six to 12 months with early syphilis and 12 to 24 months in late syphilis.[7] A recent pilot study examining the efficacy of azithromycin for the treatment of early syphilis demonstrated cure rates for a single benzathine penicillin injection of 86% and 100% at three and nine months, respectively, compared with 88% and 100% for 2g single-dose oral azithromycin.[10]

Syphilis rates in South America differ by country and population, [11,12,13,14,15,16] from 28% among female sex workers in urban Guyana[17] and 13% among female sex workers in Buenos Aires,[18] to 3% among pregnant women in Brazil[19] and 1% among donated blood in Ecuador, Chile, and Venezuela. [20]

Likewise, estimates of syphilis prevalence in Peru vary widely; 1.0% among blood donors,[21] 1.7% among young job applicants and students,[22] 3.2% among unregistered prostitutes,[23] 16.0% among men who have sex with men (MSM),[24] and 18% among HIV positive men.[25] While total visits to private clinics for syphilis have not appreciably increased for the past 15 years, [26] the number of reported cases of congenital syphilis increased from 266 to 629 between 1999 and 2000.[21]

The rate of HIV infection in Peru among the general population is 0.2%,[21] but rates as high as 18.5% have been found in populations of MSM.[24] Because 95% of all HIV infections in Peru are transmitted sexually,[21]understanding the prevalence of syphilis and its facultative relationship with HIV infection is that much more important. As such, syphilis remains a substantial public health problem in Peru, particularly among high-risk populations, underscoring the need for effective surveillance and treatment.

As part of a large, community-based, multinational STD/HIV prevention trial, we explored the relationship of treatment modality, HIV status, and initial nontreponemal antibody titer to serological response to syphilis treatment.

Materials and Methods

Study Design and Subjects

The NIMH HIV/STD Collaborative Prevention Trial was a cohort study conducted in 3 large cities in Peru among men and women aged 18-40. Each city was made up of a number of sites based on their respective populations: Lima (20 sites), Chiclayo (6), and Trujillo (4).

Recruitment was conducted (May – July, 2003) in micro-venues of low-income neighborhoods, such as bars, pool halls, soccer fields, and street corners where men and women, who were part of our target population at high risk for HIV and STDs, congregate. Study recruiters screened potential participants among patrons at the micro-venues and surroundings. To qualify for the study, participants aged 18 to 40 had to live in the neighborhood and frequent the micro-venues at least twice a week. One out of every three individuals who qualified was asked to participate; an appointment was made for an interview and specimen collection of willing participants. The sample size was 50 participants from each of the 30 venues.

Data Collection

Temporary offices were established in each of the venues to accommodate study participants who were assigned study appointments. After signing the informed consent document, participants were interviewed, submitted blood samples for biological testing, underwent assessment for current STD symptoms, and received counseling and educational materials related to STDs. All participants were given 25 soles (approximately U.S. $7) for their participation.

Approximately 15mL of venous blood was collected for testing for HIV infection, syphilis, and herpes simplex virus type 2. Participants testing positive for syphilis were asked to return four, eight, and twelve

months later in order to monitor treatment efficacy through serial Rapid Plasma Reagin (RPR) nontreponemal antibody titer measurements.

Laboratory Testing
All laboratory specimens were transported to the United States Naval Medical Research Center Detachment in Lima, Peru for testing, a laboratory site dedicated to biological testing affiliated with U.S. and approved Peruvian academic institutions. All protocols strictly adhered to CDC and Naval standards for quality control and personnel training, and testing kits were utilized according to the manufacturers' specifications. Genetic Systems HIV-1/HIV-2 Peptide EIA (Bio-Rad, Hercules, CA) was used to screen for the presence of antibodies to HIV subtype 1 and 2 with positive samples confirmed by western blot identification of HIV-1 antibodies (Genetic Systems; Bio-Rad). RPR-nosticon II Rapid Plasma Reagin kits (Shield Diagnostics, Dundee, UK) were used to screen for *T. pallidum* with reactive titers confirmed by Serodia-TPPA (Fujirebio Diagnostics Inc, Toyko, Japan) and deemed positive for syphilis if reactive. Test results were double-checked and non-diagnostic assays were repeated. All laboratory data was sent to the Research Triangle Institute (Research Triangle Park, NC) for data security, verification, blinding, and summation.

Treatment, Cure, and Re-Infection
Participants with reactive Treponema pallidum Particle Agglutination (TPPA) tests were referred for treatment and encouraged to bring in or refer sexual partners for treatment at any time and at the study's expense. Due to confidentiality, the sexual partners of infected participants were not actively sought out or otherwise contacted unless the participant requested such service, though they were encouraged to be notified and treated in any case. We treated syphilis cases with a single 2.4 million unit (MU) intramuscular injection of penicillin benzathine G or 100mg of doxycycline by mouth twice daily for two weeks if participants reported a penicillin allergy or refused the injection. Those refusing or unable to give consent for treatment were offered treatment again at the four, eight, and twelve-month follow-up appointments. If the participant did not have a sufficient drop in their RPR titer at the four, eight, or twelve month follow-ups, they were treated with a single course of weekly 2.4 MU intramuscular injections of penicillin benzathine G for three weeks or 100mg of doxycycline by mouth twice daily for four weeks. Those with titers still not dropping adequately to be considered successfully treated after the more extensive regimen had surveillance titers drawn at successive follow up appointments were observed but not offered further treatment.

We defined successful treatment as a four-fold decline in RPR titer from pre-treatment levels or titer conversion from reactive to non-reactive at any time during the twelve months. A participant was considered to have been re-infected if the criteria for successful treatment were met and then at the eight or twelve month follow up appointments they were found to have had a four-fold RPR titer increase from its lowest level or converted from non-reactive to reactive. Treatment failure occurred if participants at no time experienced a four-fold decline in RPR titer or failed to convert a 1:4 or 1:2 titer to non-reactive despite receiving both a short and long course of antibiotics.

Data Analysis
Statistical comparisons between outcomes were made with chi-square tests and Fisher exact test when appropriate. Results are presented as odds ratios (OR) with 95% confidence intervals (CI). All p-values are two-tailed and considered significant if ≤ 0.05. Epi Info 3.01 (Centers for Disease Control and Prevention, Atlanta, GA) was used for all statistical analyses.

Results
Of the 1261 participants screened in Lima, there were 96 cases (7.6%) of asymptomatic syphilis confirmed by TPPA, of which 76 (79.2%) completed follow up by also returning for a repeat RPR titer measurement one year later. Among those not completing the study, eight were treated with at least one course of

antibiotics, two were successfully treated, three were lost to follow up secondary to hospitalizations or death, and the remainder either refused treatment or failed to return to the initial encounter for their results and treatment. Five participants (5.2%) brought partners to be treated.

After the first screening, there were 14 new cases of syphilis throughout the year, representing 1.2% (14/1165) of the study population who initially tested negative for syphilis, and 41 cases of re-infection (42.7% of syphilis cases) for an annual incidence of 4.7%. 21.4% (3/14) of the new cases were participants co-infected with HIV, and new cases were associated with HIV infection (p=0.015, OR 7.12 CI 1.23-28.18). There was no association between risk of re-infection or initial RPR titer.

The syphilis cure rate was 93.4% (71/76); of the five treatment failures, one had concurrent HIV infection. The cure rate among those with initial RPR titers of ≥1:16 and ≤1:8 were 100% and 86.8%, respectively (p=0.054). By treatment modality, the cure rates for penicillin and doxycycline were 94.8% and 88.8%, respectively (p=0.59, OR=2.29, CI 0.17-21.60; Table 1). Four months after the initial treatment, 17.2% (14/81) of participants had not been successfully treated and were given additional treatment (Table 2); this was necessary more often for those with initial RPR titers ≤1:8 than those with titers ≥1:16 (26.2% vs. 7.7%; p=0.028, OR=4.26, CI 0.99-25.47). The additional treatment was successful for cases with titers ≥1:16 (2/2) but only for 30% (3/10) of those with titers ≤1:8 (p=0.15). While there was no significant difference in the need for additional treatment by initial treatment modality, none of the participants given four weeks of doxycycline were successfully treated (0/3), whereas 55.5% (5/9) of those receiving 3 penicillin injections were treated successfully (p=0.205).

The prevalence of HIV infection in this study was 15.6% (15/96) among syphilis cases and 3.7% (43/1165) among those without syphilis, and was strongly associated with syphilis infection (p<0.001, RR 4.24, CI 2.45-7.35). Of the 15 syphilis cases with concurrent HIV infection, 11 had complete follow-up data available and 10 were successfully treated (8/9 penicillin, 2/2 doxycycline) for a rate of 90.9%, compared with 93.8% for HIV negative participants (Table 3). Two syphilis cases, both with HIV infection, had an initial RPR titer of >1:512, the maximal quantitative dilution calculated in the laboratory; one was successfully treated and the other had a post-treatment titer of 1:256 before being hospitalized and lost to follow-up.

Four cases of syphilis occurred in women; two had initial RPR titers of 1:18 and two 1:16, none were co-infected with HIV, and all were treated successfully with penicillin, though two were re-infected.

Discussion

Syphilis was common in this population and strongly associated with HIV infection, corroborating findings reported by others.[27,28,29] While overall treatment success rates were high, we found that syphilis cases with an initial non-treponemal titers ≤1:8 were less often successfully treated one year after treatment with penicillin or doxycycline than those with titers of ≥1:16. Cases with low titers also required additional treatment more frequently secondary to lack of response to a single course of therapy. Finally, the high syphilis incidence and rate of re-infection in this high-risk population coupled with a very low rate of partner notification make adequate prevention and treatment a public health priority.

Primary, secondary, and early-latent syphilis are collectively termed early syphilis. Cases of syphilis without signs or symptoms are considered latent syphilis; differentiating early-latent and late-latent syphilis requires establishment of infection within the past year. This is an important distinction from a treatment point of view, as the current treatment recommendation for late-latent syphilis is three weekly penicillin injections compared to a single injection for early syphilis. The rationale for this difference is that the metabolism of *T. pallidum* changes over time, dividing less frequently, necessitating increased duration of antibiotic activity.[30]

The relationship of nontreponemal serological titers, such as the Rapid Plasma Reagin (RPR) or Venereal Disease Research Lab (VDRL) tests, to syphilis progression and treatment is complex and further complicated by host immune response, autoimmune and rheumatologic diseases, previous syphilis infection, and HIV infection. The natural history of serologic titers normally dictates a gradual rise with the onset of infection, not always detectable while symptomatic, until a peak months later correlated with the onset of secondary syphilis, after which time the titers decrease throughout the host lifetime.[31] As such, low serological titers are often correlated with latent infection.

There exist several possible explanations for the difference in treatment success rates and the need for additional treatment between patients with high- and low-titers in this study. Because it was conducted outside of a clinical setting, physical examinations were not carried out and a reliable history of painless genital ulcers was not elicited for the purposes of syphilis stage diagnosis. Thus, by treating each participant as having early syphilis, with only a single course of penicillin or doxycycline, late-latent syphilis cases may have been under-treated. Further, titers of those with latent syphilis or a history of previously treated syphilis infection are believed to decrease more slowly than those without such infection histories, potentially making one year an inadequate amount of time to definitively evaluate treatment response.[7,32,33] Finally, firm conclusions regarding differences in treatment modality cannot be drawn from this study, especially since, while compliance with penicillin injections was assured, the study depended on the participants to complete the appropriate courses of doxycycline at home, where the regimen was more complicated and observed therapy not feasible.

HIV infection was highly associated with the presence of syphilis in this population, and the cure rate was comparable to that of participants without HIV infection. The serological host response to syphilis infection and treatment in HIV-positive individuals is varied, with the literature offering conflicting findings. Nontreponemal antibody titers among individuals with both syphilis and HIV infection have been shown to be higher than those without HIV infection[34] and to decrease more slowly with therapy despite adequate clinical improvement.[35,36,37] Conversely, patients with later-stage HIV infection have shown a diminished or absent serologic response to *T. pallidum*[38,39] and a tendency to lose reactivity after efficacious treatment.[40,41] The response of treponemal tests (FTA-ABS or MHA-TP) in HIV-positive individuals seems to be less varied,[42] though HIV infection may account for some "false positive" nontreponemal tests in which treponemal-specific tests do not confirm infection.[43] Overall, despite potential fluctuations in serological titers in HIV-positive populations, the clinical response of syphilis to appropriate treatment appears to be sufficient, though increased vigilance for treatment failure is necessary given the potential consequences.

This study demonstrated an extremely high rate of participant re-infection with syphilis after successful treatment, the cause of which is surely multifaceted. This population was chosen for study in part due to its relatively high risk of acquiring STDs, and so some amount of re-infection from continued high-risk behavior is to be expected. Additionally, the utility of any interventional or educational campaign is dependent upon the ability of the target population easily understand the problem and put realistic corrective measures into place with proper training, a property which can not be assured in this study since, despite treatment and counseling, such points were never directly measured. Object data does show, however that the risk of re-infection in this study was independent of sex, HIV status, and initial RPR titer. Finally, the dearth of partner treatment by the study points to an overall deficiency of partner notification. This can be an overwhelming problem since no number or combination of medication regimens can be protective against syphilis if a regular sexual partner is infected and does not seek treatment. We can not be sure whether study participants understood the importance of notifying their partners, went through with the notification, or brought them for treatment elsewhere, but ongoing work in this particular area points to low overall levels of

partner notification in cases of sexually transmitted diseases, with embarrassment, multiple casual partners, and fear of breaking up endorsed most frequently as reasons for not notifying (data unpublished).

A recent review of international syphilis treatment guidelines, as well as the evidence in support of such guidelines, shows that despite evaluating the same studies, the CDC and European health authorities differ in first-line recommended treatment regimens.[9] The UK, for example, treats early syphilis with daily intramuscular injections of 750 mg of procaine penicillin for 10 days due to concerns about benzathine penicillin failure rates, particularly among pregnant women and the immunocompromised, and its low CSF penetration; benzathine penicillin is second-line and given in two 2.4 MU intramuscular injections 1 week apart.[44] Treatment of latent syphilis follows the same pattern, with the UK extending the injections of procaine penicillin to 17 days and maintaining 3 weekly benzathine penicillin injections as second-line.[45] Official European guidelines, despite the diversity of individual national recommendations, largely conform to those of the CDC.[46]

Several areas of research in the field of syphilis management have yet to be sufficiently explored. This study raises questions related to the efficacy of a single course of penicillin or doxycycline for patients with low initial nontreponemal titers and, given the low treatment response rate, the role of doxycycline in the treatment of latent syphilis. The efficacies of alternative therapies, including single-dose azithromycin for early syphilis, are currently being explored. Finally, work is ongoing to delineate the most effective treatment regimens for special subsets of syphilis patients, such as those with HIV infection or neurosyphilis.

This study was not without its limitations. Despite following participants for one year, some decrease in RPR titers could continue beyond this time, allowing fewer participants to have "failed" treatment. The overall number of syphilis cases in this study was high considering it was a cross-sectional community-based study, but still lacked sufficient numbers to carry out more sensitive analysis of response to the different medical therapies according to titer. As an epidemiological undertaking, rather than a clinical one, medical histories, physical examinations, and other laboratory tests were not utilized to better characterize participant disease, thereby precluding definitive diagnosis of late or neurosyphilis. Finally, if such interventions are to have a real and sustainable positive impact on the study population, a better mechanism for partner notification and treatment must be considered.

Syphilis was found to be common in this high-risk population, often concurrent with HIV infection. Participants with low initial RPR titers may have been less frequently successfully treated one year after treatment than those with higher titers. Since lower titers often signify older infection, these findings support current CDC and UK guidelines for syphilis treatment, which emphasize extended treatment regimens for suspected late syphilis. The high rate of re-infection and low rate of partner treatment demonstrated by this study underscore the need for effective partner notification and treatment strategies to combat the spread of HIV and other STDs.

TABLE 1

Syphilis Treatment Success Rate Percentages (n/N) by Baseline Rapid Plasma Reagin (RPR) Titer and Treatment Modality

Titer	Penicillin	Doxycycline	Total
≤ 1:8	88 (22/25)	84.6 (11/13)	86.8 (33/38)
≥ 1:16	100 (33/33)	100 (5/5)	100 (38/38)
Total	94.8 (55/58)	88.8 (16/18)	93.4 (71/76)

TABLE 2

Percentage of Syphilis Cases (n/N) Requiring Additional Treatment by Baseline Rapid Plasma Reagin (RPR) Titer and Primary Treatment Modality

Titer	Penicillin	Doxycycline	Total
≤ 1:8	23.3 (7/30)	33.3 (4/12)	26.2 (11/42)
≥ 1:16	8.8 (3/34)	0 (0/5)	7.7 (3/39)
Total	15.6 (10/64)	23.5 (4/17)	17.2 (14/81)

TABLE 3

Syphilis Treatment Success Rate Percentages (n/N) by Baseline Rapid Plasma Reagin (RPR) Titer and HIV Status

Titer	HIV +	HIV -	Total
≤ 1:8	75.0 (3/4)	88.2 (30/34)	86.8 (33/38)
≥ 1:16	100 (7/7)	100 (31/31)	100 (38/38)
Total	90.9 (10/11)	93.8 (61/65)	93.4 (71/76)

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