

Comparisons of treatment responses of early syphilis to 1.0 g single-dose ceftriaxone plus doxycycline versus 2.4 MU single-dose benzathine penicillin G plus doxycycline in people living with HIV

Background

While improvement of access to antiretrovirals for HIV treatment and prevention continue and the case numbers of newly diagnosed HIV infection have stabilized or decreased, sexually transmitted infections (STIs) have been increasing worldwide since late 1990s.¹ Concurrent with the increases in established STIs are emerging epidemics of non-classical sexually transmissible pathogens and co-infections with more than one STI. Furthermore, increases in antimicrobial resistance have limited therapeutic options for STIs, particularly *Neisseria gonorrhoeae* and *Mycoplasma genitalium* infections.²

In Taiwan, we had experienced epidemics and outbreaks of non-classical STIs predominantly affecting men who have sex with men (MSM) living with HIV, such as shigellosis since 2015, acute hepatitis A during 2015-2017, and acute hepatitis C since 2017.³⁻⁵ In addition, syphilis and gonorrhea have been increasingly reported as notifiable diseases over the past decade, while the incidence of HIV infection has significantly decreased since 2018. To facilitate early detection and treatment of individuals with STIs, we have established regular screening for chlamydia, gonorrhea, and syphilis in people living with HIV (PLWH) and MSM receiving pre-exposure prophylaxis (PrEP) in our hospital. In our prospective study investigating the prevalence of STIs among at-risk PLWH, the prevalence of *Chlamydia trachomatis* and *N. gonorrhoeae* was 24.7% and 12.1%, respectively. Surprisingly, we found high rates of *C. trachomatis* and/or *N. gonorrhoeae* co-infections in PLWH with recent hepatitis C virus (HCV) infection (50%), HBsAg positivity (44%), and early syphilis (36%).⁶ Although *C. trachomatis* co-infections have been reported in 20-42% of individuals with *N. gonorrhoeae* infections, few studies document the prevalence of *C. trachomatis* and *N. gonorrhoeae* co-infections among patients with syphilis and viral hepatitis. However, a recent sub-study nested in a randomized controlled trial comparing the efficacy of single dose versus 3 weekly doses of benzathine penicillin G (BPG) for early syphilis also showed a high rate of sexually transmitted co-infections (27%) in patients with early syphilis, including *C. trachomatis*, *N. gonorrhoeae*, and non-gonococcal urethritis.⁷ The findings highlight the importance of integrating bacterial STI screening and treatment into management of early syphilis.

Benzathine penicillin G (BPG) (2.4 million units [MU] intramuscularly once) has been the recommended treatment for early syphilis, but is not effective for treating *C. trachomatis* and *N. gonorrhoeae* co-infections.⁸ The recurrence of BPG shortages has also created an urgent need to identify other alternative treatment that is as effective as BPG for treating early syphilis. Ceftriaxone and doxycycline have been the alternative treatments for

early syphilis; in addition, ceftriaxone (500 mg intramuscularly once) is effective for treating *N. gonorrhoeae* infection, and doxycycline (100 mg orally twice daily for 7 days) effective for treating *C. trachomatis* infection.⁸ However, the current suggested dose of ceftriaxone for early syphilis (1 g intramuscularly or intravenously once daily for 10-14 days) is inconvenient as an outpatient treatment option. Ceftriaxone is long-acting, with treponemacidal levels remaining at 24 hours following injection. According to a small clinical trial, single-dose ceftriaxone achieved a serological response similar to that of BPG.⁹ Considering the high rate of sexually transmitted co-infections, combination therapy of single-dose ceftriaxone plus 7-day doxycycline for early syphilis may provide convenience and benefit to treatment of *N. gonorrhoeae* and *C. trachomatis* co-infections at a single clinic encounter. In the present study, we aim to compare the efficacy of ceftriaxone plus doxycycline versus BPG plus doxycycline as the treatment for early syphilis among PLWH.

Methods

1. Study design: randomized controlled, open-label, non-inferiority study

2. Study site: infectious diseases clinics at the National Taiwan University Hospital (NTUH)

3. Study duration: 2022-2025

4. Enrolled criteria:

(1) PLWH aged 20 years or more

(2) PLWH with early syphilis (i.e. primary, secondary, or early latent syphilis), confirmed by a positive rapid plasma reagin (RPR) titer with a reactive *Treponema pallidum* particle agglutination (TPPA) assay.

(3) PLWH has provided informed consent

*A participant with repeated syphilis can be repeated enrolled after signing an informed consent if the previous episode of early syphilis was successfully treated with achieving at least a 4-fold decrease in RPR titers and 48-week follow-up is completed.

5. Exclusion criteria:

(1) PLWH with RPR titers of less than 4

(2) Exposure to antibiotics with activity against *T. pallidum*, such as penicillins, third-generation cepheems, doxycycline, or macrolides, within the preceding 4 weeks

(3) A known or suspected infection other than syphilis requiring additional treatment with an antimicrobial active against *T. pallidum*

(4) A history of intolerance to penicillin, ceftriaxone, or doxycycline

(5) Pregnancy

6. Study procedures: participants will be randomized in a 1:1 ratio to receive ceftriaxone plus doxycycline or BPG plus doxycycline. Randomization will be stratified by the stage of syphilis and RPR titer at baseline.

Arm 1: single-dose ceftriaxone (1g intramuscularly once) plus doxycycline (100 mg orally twice daily for 7 days)

Arm 2: single-dose BPG (2.4 MU intramuscularly once) plus doxycycline (100 mg orally twice daily for 7 days)

7. Baseline and follow-up testing:

Proof of concept phase: (enrolled 50 participants in each group)

- (1) Questionnaire for socio-epidemiologic characteristics and sexual history at baseline and weeks 24, 48
- (2) Serum RPR at baseline and weeks 4, 12, 24, 36, and 48
- (3) *T. pallidum* PCR (TP-PCR) for self-collected specimens from oral rinse, urethral, and rectal sites at baseline, weeks 1, 2, 3, 4, 12, 24, 36, and 48 (specimens collected in weeks 1, 2, and 3 were optional)
- (4) Bacterial STIs (*C. trachomatis*, *N. gonorrhoeae*, *Mycoplasma genitalium*, *M. hominis*, *Ureaplasma urealyticum*, *U. parvum*) for self-collected specimens from oral rinse, urethral, and rectal sites at baseline and week 4
- (5) Resistance-associated mutations (RAM) of *T. pallidum* and other sexually transmitted bacteria at baseline

	Baseline	Wk1 (±2 days)	Wk2 (±2 days)	Wk3 (±2 days)	Wk4 (±2 days)	Wk12 (±2 weeks)	Wk24 (±2 weeks)	Wk36 (±2 weeks)	Wk48 (±2 weeks)
Questionnaire	v						v		v
Doxycycline compliance record		v							
RPR	v				v	v	v	v	v
TP-PCR	v	v	v	v	v	v	v	v	v
		(optional)							
Bacterial STIs	v				v				
RAM	v								

Continuation phase: (enrolled 100 participants in each group)

- (1) Questionnaire for socio-epidemiologic characteristics and sexual history at baseline and

weeks 24, 48

(2) Serum RPR at baseline and weeks 4, 12, 24, 36, and 48

(3) *T. pallidum* PCR (TP-PCR) for self-collected specimens from oral rinse, urethral, and rectal sites at baseline, weeks 4, 12, 24, 36, and 48

(4) Bacterial STIs (*C. trachomatis*, *N. gonorrhoeae*, *M. genitalium*, *M. hominis*, *U. urealyticum*, *U. parvum*) for self-collected specimens from oral rinse, urethral, and rectal sites at baseline and week 4

(5) Resistance-associated mutations (RAM) of *T. pallidum* and other sexually transmitted bacteria at baseline

	Baseline	Wk1 (±2 days)	Wk2 (±2 days)	Wk3 (±2 days)	Wk4 (±2 days)	Wk12 (±2 weeks)	Wk24 (±2 weeks)	Wk36 (±2 weeks)	Wk48 (±2 weeks)
Questionnaire	v						v		v
Doxycycline compliance record		v							
RPR	v				v	v	v	v	v
TP-PCR	v				v	v	v	v	v
Bacterial STIs	v				v				
RAM	v								

8. Duration of study: Participants will be followed for at least 48 weeks.

9. Primary outcome:

Serologic response at month 6 (defined as either a 4-fold or greater decline in RPR titer compared to baseline or being RPR-nonreactive)

10. Secondary outcomes:

(1) Microbiologic response of syphilis (defined as *T. pallidum* PCR Ct value >38) at week 4

(2) Microbiologic response of bacterial STIs (defined as negative PCR results) at week 4

(3) Serologic response at months 3 and 12

(4) Safety of study treatment recorded by using a diary (all adverse events will be coded and graded according to Common Terminology Criteria for Adverse Events [CTCAE] v4.0.)

(5) Adherence evaluation (the noting of tablet intake in the diary for the 7 days of the treatment period)

11. Futility stopping rules:

(1) In the proof-of-concept phase, microbiologic and/or serologic response of syphilis

(microbiologic response was defined as *T. pallidum* PCR Ct value >38, and serologic response was defined as at least a 4-fold decrease in RPR titers) at week 4 will be compared between study groups. The non-inferiority margin of 10% will be used as the futility stopping rule in the proof-of-concept phase.

(2) Participants with treatment failure at week 24 will receive standard-of-care treatment (BPG 2.4 MU intramuscularly once). Treatment failure is defined as at least a 4-fold increase in RPR titers compared with the baseline RPR or at least a 4-fold increase in RPR titers after achieving serologic response during follow-up. Participants who received additional treatment with an antimicrobial active against *T. pallidum* (i.e. penicillins, third-generation cepheems, doxycycline, or macrolides) will also be excluded from the study.

(3) The continuation phase will be conducted only if microbiologic response of syphilis is non-inferior in the ceftriaxone plus doxycycline group compared with the BPG plus doxycycline group.

Laboratory investigations

1. *T. pallidum* PCR:

T. pallidum PCR will be performed on specimens from oral rinse, urine, rectal sites, blood, and lesions of syphilis. Treponemal DNA will be extracted with the QIAamp DNA mini Kit. The diagnostic PCR assay will be used to amplify the 47 kDa protein membrane gene (*tpp47*).

2. Bacterial STIs screening:

The screening for bacterial STIs, including *C. trachomatis*, *N. gonorrhoeae*, *Trichomonas vaginalis*, *Mycoplasma* spp., and *Ureaplasma* spp.) will be performed on self-collected specimens from oral rinse, urethral, and rectal sites with the use of multiplex real-time PCR assay on an automated system.

3. Resistance-associated mutation of *T. pallidum*

Single-nucleotide polymorphisms (SNPs) will be determined at positions A2058G or A2059G of the 23S rRNA gene, as well as positions 965–967, 926–928, 939 and 1058 of the 16S rRNA gene.

Statistical analysis

According to the previous randomized controlled, open-label study conducted by Cao Y et al, the serological response of early syphilis at 6 months of follow up was observed in 90.2% in ceftriaxone group and 78.0% in BPG group.¹⁰ Therefore, at least 150 participants would be

required for each treatment group to detect a between-group difference, with a two-sided significance level of 5% and a power of 80%. The intention-to-treat population is defined as all patients who undergo randomization except for those who do not provide consent for the use of their data. The analyses will be performed through the FDA snapshot algorithm in the ITT population, which categorizes outcomes into success, failure, and no data in window. The Categorical variables will be compared by Fisher's exact test or Chi-square test. Non-categorical variables will be compared by Mann-Whitney U test. Factors with P value ≤ 0.2 , or with biological significance will be included for multivariate analysis. Logistic regression analysis will be used to determine the factors associated with serologic responses after treatment. All comparisons will be two-tailed and a P value <0.05 is considered significant. All statistical analyses were performed with the use of STATA software version 17.0 (Stata Corporation, College Station, TX).

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