

Study Protocol

Date: 2025/09/30

1. **Study title:** 500-mg versus 1000-mg ceftriaxone for uncomplicated gonorrhea Infection: A noninferiority randomized controlled trial

2. **Study rationale and Background**

Gonorrhea has become the second most common bacterial STI worldwide.¹ In Taiwan, gonorrhea has been increasingly reported as a notifiable disease over the past decade, with 8257 confirmed cases reported in 2023.² Through regular screening, we previously identified a high infection rate of *Neisseria gonorrhoeae* (12.1%) among sexually active, HIV-positive MSM who presented with syphilis or symptoms suggestive of STIs.³ Efforts to control the epidemic are critically important.

Ceftriaxone remains the first-line antibiotic for the empirical treatment of gonorrhea. In recent years, ceftriaxone-resistant strains have emerged in several countries.⁴ The prevalence of *N. gonorrhoeae* with a ceftriaxone MIC >0.03 µg/mL rose in the UK by 2018, prompting the UK and European guidelines to adopt 1000-mg ceftriaxone monotherapy.^{5,6} The latest 2024 WHO guideline now recommends 1000-mg ceftriaxone for gonorrhea treatment.⁷

In Taiwan, unlike neighboring countries, the prevalence of *N. gonorrhoeae* with decreased susceptibility to ceftriaxone, as defined by WHO criteria (MIC ≥0.125 µg/mL), remains below 5%.⁸ The 2023 Taiwan guidelines currently recommend ceftriaxone monotherapy at a 500-mg dose.⁹ The efficacy of a 500-mg ceftriaxone dose in settings of a low prevalence of ceftriaxone-resistant *N. gonorrhoeae* remains unclear. As part of our sub-study within a prospective study assessing the prevalence of concomitant bacterial STIs among at-risk people with HIV (PWH) and PrEP users, we retrospectively evaluated the treatment response of gonorrhea to different doses of ceftriaxone. We found that both the 500-mg and 1000-mg ceftriaxone doses demonstrated similarly high effectiveness in treating uncomplicated gonorrhea infections (UGIs) (90.6% [173/191] vs 88.9% [8/9], P>0.999) (unpublished data).

There are several theoretical advantages of using a 500-mg dose of ceftriaxone over a 1000-mg dose. In Taiwan, the commercially available ceftriaxone sodium is supplied in 500-mg vials (Sintrix injection, 500-mg /vial). Treating UGIs with a single 500-mg vial is more convenient during drug administration than using two vials and is also less expensive. Ceftriaxone is known to cause injection site pain when administered intramuscularly. A lower dose may result in less pain during treatment, potentially improving patient willingness to receive therapy.

3. **Study objectives**

1. Primary objective: to compare the treatment responses of UGIs between 500-mg and 1000-mg doses of ceftriaxone
2. Secondary objectives:
 - i. To compare the symptom duration in the subgroup of patients who have baseline symptoms attributable to UGIs between 500-mg and 1000-mg doses of ceftriaxone.
 - ii. To compare the treatment-related adverse events after treatment between 500-mg and 1000-mg doses of ceftriaxone.

- iii. To estimate the prevalence of *N. gonorrhoeae* strains with resistance-associated mutations.
- iv. To clarify the association of treatment responses of UGIs with the minimal inhibitory concentration (MIC) of the *N. gonorrhoeae* isolates.
- v. To clarify the association of treatment responses of UGIs with *N. gonorrhoeae* multi-antigen sequence typing (NG-MAST).
- vi. To clarify the association of treatment responses of UGIs with resistance-associated mutations for extended-spectrum cephalosporins (ESCs) of the *N. gonorrhoeae* strains.

4. Study design

1. Methodology: multicenter randomized controlled non-inferiority trial
2. Study sites: National Taiwan University Hospital (NTUH) Yunlin Branch, NTUH Main Branch, NTUH Hsin-Chu Branch, Far Eastern Memorial Hospital
3. Study endpoints
 - i. Primary endpoint: The proportion of participants with successful treatment of UGI, defined as a NAAT-negative and, if applicable, a culture-negative result of the primary anatomic sites of infection 7-14 days after treatment; a culture-negative result is required only if *N. gonorrhoeae* culture was positive at the screening visit
 - ii. Exploratory secondary endpoints:
 1. Symptom duration, including dysuria, genital discharge, sore throat, anorectal pain, rectal bleeding, and rectal discharge, in the subgroup of patients who have baseline symptoms attributable to UGIs
 2. Treatment-related adverse events until 14 days after treatment
 3. MIC of ceftriaxone of isolates at screening and test-of-cure (TOC) visits
 4. NG-MAST at screening and TOC visits
 5. Gene mutations associated with drug resistance for extended-spectrum cephalosporins at screening and TOC visits
4. Randomization and stratification
 - i. Randomization will be conducted by a central randomization center using a computer-generated block randomization sequence to ensure a 1:1 allocation ratio between the two study arms.
 - ii. Randomization will be stratified by study site.
5. Study intervention: Either a single intramuscular dose of 500 mg or 1000 mg of ceftriaxone will be administered. Details of the study medications are provided in the 'Study intervention' section, and the schedule of clinic visits is outlined in the 'Study assessment and procedure' section.
6. Study period: Participant recruitment and follow-up will take place starting from January 1, 2026, to December 31, 2028.

5. Inclusion/Exclusion criteria

1. Inclusion criteria:
 - i. 18 years or older man, and
 - ii. having oropharyngeal, anorectal or urogenital *N. gonorrhoeae* infection confirmed by culture or a positive NAAT
2. Exclusion criteria:

- i. Suspicion of a complicated *N. gonorrhoeae* infection. Complicated *N. gonorrhoeae* infection is defined as the presence of epididymitis, arthritis, bacteremia, conjunctivitis, or disseminated gonococcal infection.
- ii. Use of systemic cephalosporins, doxycycline, or azithromycin at the screening visit or within 30 days prior to the enrollment visit is prohibited. Participants who require immediate antibiotic treatment at the screening visit, based on clinician judgment or shared decision-making with the participant, without awaiting PCR or culture confirmation, will be excluded from the study. Use of doxycycline for post-exposure prophylaxis (Doxy-PEP) is permitted.
- iii. A NAAT test positive for *C. trachomatis* or *M. genitalium* at the screening visit.
- iv. A history of allergy or intolerance to ceftriaxone, penicillins, or lidocaine, in alignment with the contraindications listed in the Sintrix (ceftriaxone) injection package insert. Allergy or intolerance is defined as the occurrence of adverse symptoms following antibiotic administration, including but not limited to skin rash, pruritus, dyspnea, angioedema, anaphylaxis, dizziness, nausea, vomiting, headache, or diarrhea. Laboratory abnormalities include cytopenias, eosinophilia, and elevated levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, or creatinine. Penicillins include natural penicillins (e.g., penicillin G), aminopenicillins (e.g., amoxicillin, ampicillin), penicillinase-resistant penicillins (e.g., oxacillin, dicloxacillin), extended-spectrum penicillins (e.g., piperacillin), and other agents classified within the penicillin class.

6. Study intervention

- 1. The investigational medications for both study arms will be administered by a trained nurse. The injection will be delivered into the gluteal muscle of one buttock. The volume and concentration of lidocaine used are consistent with those specified in the package insert for Sintrix (ceftriaxone) injection.¹⁰
 - i. Arm 1: Single-dose ceftriaxone 500-mg intramuscular injection, dissolved in 2 mL of 1% lidocaine hydrochloride solution
 - ii. Arm 2: Single-dose ceftriaxone 1000-mg intramuscular injection, dissolved in 3.5 mL of 1% lidocaine hydrochloride solution

- 7. **Concomitant therapy/medications:** The use of systemic cephalosporins, doxycycline, or azithromycin is prohibited starting from the screening visit to the TOC visit.

- 8. **Permanent discontinuation/Withdrawal criteria:** Participants must withdraw from the study under the following circumstances:

- 1. Suspicion of a complicated *N. gonorrhoeae* infection, such as epididymitis, arthritis, bacteremia, conjunctivitis, or disseminated gonococcal infection;
- 2. Requirement for treatment with cephalosporins, doxycycline, or azithromycin due to other infections.

After withdrawal, participants may choose how their previously collected specimens will be handled and decide whether to allow the principal investigator to continue collecting their data.

9. Study assessment and procedure

- 1. Visit 1 (the screening visit)
 - i. Bacterial STIs: self-collected from oral rinse, urethral, and rectal sites, and subsequently subjected to a multiplex real-time PCR assay (Allplex™ STI Essential Assay; Seegene Inc, Seoul, Korea) to identify *C. trachomatis*, *N. gonorrhoeae*,

and *M. genitalium*. For specimen collection, participants will swish and gargle 10 mL of sterile water for 15 seconds to obtain oral rinse samples, insert a blind swab 3-5 cm into the anal canal and rotate to obtain rectal site samples, and insert a swab 1 cm into the urethra and rotate to obtain urethral samples. Clear instructions will be provided by a trained research staff to ensure proper self-collection. Samples positive for *N. gonorrhoeae* will undergo NG-MAST and resistance-associated mutations testings (*penA* and *porB*) using in-house PCR assays.

- ii. For symptomatic participants, specimens will be collected from the symptomatic site by the clinician and subsequently cultured on GC agar plates. Isolates that test positive for *N. gonorrhoeae* will undergo MIC testing with breakpoints according to the CLSI guideline.

2. Visit 2 (the enrollment visit, 0-30 days after visit 1)

- i. Self-collection for bacterial STIs will be repeated using the same method as conducted during the screening visit.
- ii. Study drug from one of the arms will be administered by a trained nurse. Participants will be required to remain under observation in the clinic for at least 30 minutes following drug administration to monitor for any immediate treatment-related adverse events.
- iii. Serum RPR test, if the participant does not have a RPR test within the past month.
- iv. HIV Combo screening test, if the participant does not have a diagnosis of HIV and does not receive HIV screening within the past 3 months.
- v. Basic clinical information will be collected, including age, height, and weight. For participants living with HIV, the most recent CD4 count and HIV viral load within the past 6 months will be recorded from medical records.
- vi. A self-administered questionnaire interview will be collected to record the socioeconomic status, clinical characteristics, signs and symptoms suggestive of STIs, previous history of STIs, sexual behaviors, use of other recreational drugs.

3. Visit 3 (the TOC visit, 7-14 days after visit 2)

- i. Self-collection for bacterial STIs will be repeated using the same method as conducted during the screening visit. This timepoint was selected based on evidence that a negative NAAT result is typically observed within several days following successful treatment of UGI.¹¹
- ii. For participants who had a positive *N. gonorrhoeae* culture at the screening visit, specimens from the previously symptomatic site will be collected by the clinician and *N. gonorrhoeae* cultures will be repeated.
- iii. A participant diary to record symptoms, adverse events and sexual behavior will be collected. Given that the intervention involves a single-dose ceftriaxone injection, adverse events are expected to occur within a few hours to several days post-administration. Therefore, a 7 to 14-day follow-up period for diary-based monitoring is considered adequate for capturing relevant adverse events.

4. Adverse events and Serious adverse events: Common side effects associated with ceftriaxone include gastrointestinal discomfort (approximately 2%), hematologic changes (approximately 2%), and skin reactions (approximately 1%). A known allergy to ceftriaxone constitutes a contraindication to its use. Urethral and rectal swab collection may cause temporary local discomfort or minor bleeding (<1%). Blood

draws may lead to temporary discomfort, bruising, bleeding, swelling (<2%), or localized infection at the puncture site (<1%). If participants experience any related discomfort, they should contact the clinician or the research staff for appropriate medical care.

10. Statistics

1. Primary hypothesis: The primary hypothesis is that 500 mg ceftriaxone is non-inferior to 1000 mg ceftriaxone in the treatment of UGIs, with a non-inferiority margin set at 8%. While previous studies and FDA guidance for gonorrhea treatment have used a 10% non-inferiority margin, this study adopts a stricter threshold for two key reasons: first, the FDA guidance is based on culture-confirmed outcomes, whereas this study uses NAAT, which may have different performance characteristics and higher sensitivity; second, given that both treatment arms are expected to achieve serologic response rates >80%, a smaller margin provides greater confidence in establishing non-inferiority..
2. Sample size calculation: Assuming that 90% of the participants in the 500-mg ceftriaxone group and 89% of those in the 1000-mg ceftriaxone group would achieve microbiologic responses (according to the previous retrospective study), we estimate that assigning 183 participants to each group provides the trial with 80% power to establish the non-inferiority of 500-mg ceftriaxone, defined as the lower limit of the two-sided 95% confidence interval exceeding -8 percentage points. To account for a 10% loss to follow-up, we aim to include 200 participants with a positive *N. gonorrhoeae* test result in each treatment group.
3. Analyses: The primary analysis will be conducted using the intention-to-treat (ITT) population, while the per-protocol (PP) population will be used for secondary analysis.
4. Missing data:
 - i. Participants who do not attend Visit 3, fail to complete bacterial STI testing at Visit 3, do not undergo *N. gonorrhoeae* culture for symptomatic sites at Visit 3, withdraw from the study due to developing complicated *N. gonorrhoeae* infection, or receiving treatment with cephalosporins, doxycycline, or azithromycin due to other infections, will be included in the ITT analysis and classified as treatment failures. These participants will be excluded from the PP analysis.
 - ii. For exploratory secondary endpoints, including symptom duration and treatment-related adverse events, analyses will be performed using the PP population. Participants who do not complete the participant's diary or fail to provide specimens required for MIC, NG-MAST, or resistance gene mutation testing will be excluded from these analyses.
5. Safety set: Participants are included if they received any doses of ceftriaxone.
6. Interim analysis: There will be no interim analysis.
7. Categorical variables are compared using Fisher's exact test or Chi-square test, while non-categorical variables are compared using the Mann-Whitney U test. All comparisons are two-tailed, and a P value <0.05 is considered significant. Statistical analyses are performed with the use of STATA software version 18.0 (Stata Corporation, College Station, TX).

11. Schedule of activities

1. for asymptomatic participants

	Visit 1	Visit 2	Visit 3
Aim of the visit	Screening	Enrollment	TOC
Timing		0-30 days after visit 1	7-14 days after visit 2
Intervention			
Ceftriaxone 500mg or 1000mg		V	
Laboratory exam			
Allplex™ STI Essential Assay	V	V	V
NG-MAST and resistance-associated mutations (for positive NAAT specimens)	V		V
Serum RPR (for participants without RPR testing within the past month)		V	
HIV Combo screening test (for non-PWH participants without HIV screening within the past 3 months)		V	
Other data			
Age, height, weight		V	
CD4 count and HIV viral load (for PWH)		V	
Self-administered questionnaire		V	
Participant diary for symptoms, adverse events and sexual behavior			V

2. Symptomatic participants who are willing to defer treatment until either culture or PCR result is available, either at their own request or based on the clinician's decision.

	Visit 1	Visit 2	Visit 3
Aim of the visit	Screening	Enrollment	TOC
Timing		0-30 days after visit 1	7-14 days after visit 2
Intervention			
Ceftriaxone 500mg or 1000mg		V	
Laboratory exam			
Allplex™ STI Essential Assay	V	V	V
NG-MAST and resistance-associated mutations (for positive NAAT specimens)	V		V
<i>N. gonorrhoeae</i> culture	V		V
MIC (for positive <i>N. gonorrhoeae</i> cultures)	V		V
Serum RPR (for participants without RPR testing within the past month)		V	
HIV Combo screening test (for non-PWH participants without HIV screening within the past 3 months)		V	
Other data			
Age, height, weight		V	
CD4 count and HIV viral load (for PWH)		V	
Self-administered questionnaire		V	
Participant diary for symptoms, adverse events and sexual behavior			V

12. Reference

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