ACADEMICAL MEDICAL CENTER (AMC) – PUBLIC HEALTH SERVICE (GGD) AMSTERDAM

New antibiotic treatment options for uncomplicated anogenital gonorrhea (NABOGO trial)

a double-blind randomized controlled non-inferiority trial

This document contains the protocol of a double-blind randomized controlled non-inferiority trial investigating registered antibiotics as new treatment options for uncomplicated anogenital gonorrhea. In this protocol the rationale and the study procedures are described in detail.

PROTOCOL TITLE: New antibiotic treatment options for uncomplicated anogenital gonorrhea infections – a double-blind randomized controlled non-inferiority trial

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	**ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee (In Dutch, ABR = Algemene Beoordeling en Registratie)**
AMC	**Academic Medical Center**
AMR	**Antimicrobial Resistance**
AE	**Adverse Event**
AR	**Adverse Reaction**
CA	**Competent Authority**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
CV	**Curriculum Vitae**
DALY	**Disability Adjusted Life Years**
DSMB	**Data Safety Monitoring Board**
eGFR	**estimated Glomerular Filtration Rate**
EMA	**European Medicines Agency**
ESC	**Extended Spectrum Cephalosporin**
EudraCT	**European Drug Regulatory Affairs Clinical Trials**
FDA	**Food and Drug Administration**
GCP	**Good Clinical Practice**
HIV	**Human Immunodeficiency Virus**
IB	**Investigator's Brochure**
IC	**Informed Consent**
IM	**Intramuscular(ly)**
IMP	**Investigational Medicinal Product**
IMPD	**Investigational Medicinal Product Dossier**
ITT	**Intention To Treat analysis**
IV	**Intravenous(ly)**
METC	**Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)**
MIC	**Minimum Inhibitory Concentration**
MITT	**Modified Intention To Treat analysis**
MSM	**Men who have sex with men**
NAAT	**Nucleic Acid Amplification Test**

Ng	***Neisseria gonorrhoeae***
NONMEM	**Non-linear mixed effects modelling**
PP	**Per Protocol analysis**
POC	**Point-of-Care (test)**
RCT	**Randomized Controlled Trial**
(S)AE	**(Serious) Adverse Event**
SPC	**Summary of Product Characteristics**
Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a funding source.**
STI	**Sexually Transmitted Infection**
SUSAR	**Suspected Unexpected Serious Adverse Reaction**
TOC	**Test of Cure**
Wbp	**Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)**
WHO	**World Health Organization**
WMO	**Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**

SUMMARY

Rationale: Antimicrobial resistance (AMR) to extended spectrum cephalosporins (ESC) in *Neisseria gonorrhoeae* (Ng) is a major public health concern. With no alternative options for the antimicrobial treatment of gonorrhea and only few new drugs in development, it is important to test existing antibiotics on their efficacy to treat gonorrhea.

Objective: This project aims to identify new treatment modalities for uncomplicated gonorrhea using the registered drugs ertapenem, fosfomycin and gentamicin.

Study design: A double-blind randomized controlled non-inferiority trial with three treatment arms.

Study population: Patients with anogenital gonorrhea visiting the sexually transmitted infection (STI) outpatient clinic of the Public Health Services Amsterdam, aged 18 years and older, both female and male, with multiple ethnic backgrounds. The highest prevalence of gonorrhea is seen among men who have sex with men (MSM).

For a substudy on the pharmacokinetics (PK) of the studied antibiotics, we aim to include 60 volunteers who will be recruited from the STI clinic, the Public Health Service and the University of Amsterdam.

Intervention: Patients will be randomly assigned to one of four treatment arms. They receive one of the following: ceftriaxone 500mg intramuscularly (IM), ertapenem 1000mg IM, gentamicin 5mg/kg IM with a maximum of 400mg (in two doses) supplemented with an oral placebo, or fosfomycin 6g oral suspension supplemented with an intramuscular placebo. Following the advice of the DSMB, we stopped administering fosfomycin and oral placebo to NABOGO participants on October 2, 2018.

Main study endpoint: The bacterial eradication capacity of study antimicrobials at the included infection site using an RNA-based Nucleic Acid Amplification Test (NAAT) 7-14 days after treatment.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: With 78 million new cases of gonorrhea in adults aged 15-49 years, gonorrhea is the second most common bacterial STI worldwide. Persistent Ng infections may cause severe reproductive tract inflammation and irreversible damage resulting in infertility. Moreover, gonorrhea increases the risk of human immunodeficiency virus (HIV) transmission. If untreatable gonorrhea becomes a reality, an increase in both the burden of disease and costs for society will be observed.

The drugs in this study are all widely-used antimicrobials. The most important side effects are gentamicin-associated nephro- and ototoxicity. However, limited research has been done on the safety of single dose intramuscular injection of gentamicin. We expect that single dose intramuscular injection will not harm young and healthy participants. For safety concerns regarding renal function after the administration of gentamicin, creatinine clearance will be monitored before and after treatment in participants of the main study. Patients with renal impairment are not eligible to take part in this study.

The burden of participation in this study includes two intramuscular injections instead of one, which is due to the large required volume for injectable gentamicin (6-10 ml). A finger prick will be performed to draw blood for a point-of-care (POC) serum creatinine test, participants will be requested to return 7-14 days after treatment for a test of cure and will be asked to keep a diary and abstain from sexual contact or condom use during the period between treatment and follow-up visit.

We aim for 60 NABOGO participants (=gonorrhea patients) to provide one blood sample (obtained by vena punction) within 30-90 minutes after treatment administration to measure the plasma concentration of the administered antibiotic.

Participants (n=60) of the PK substudy (= healthy volunteers) will receive either ceftriaxone 500mg IM (n=20), ertapenem 1000mg IM (n=20) or fosfomycin 6g PO (n=20) and will provide four blood samples within 24 hours after treatment administration to measure plasma concentration at different time points.

LIST OF MODIFICATIONS AMENDEMENT 3

No	Subject	Previous situation/description	Description of modifications	Rationale
C1	No point of care measurement of creatinine in PK substudy with volunteers	We planned to measure the creatinine serum levels with a point of care test to assess renal impairment in healthy volunteers.	Severe renal impairment remains an exclusion criterion for volunteers participating in the PK substudy. However, we propose to evaluate renal impairment by medical history rather than by assessing creatinin serum level.	A single dose of ceftriaxone 500mg IM, ertapenem 1000mg IM or fosfomycin 6g orally can safely be administered to persons with mild renal impairment (eGFR 30-50 ml/min). In this healthy volunteer population we do not expect anybody to have severe renal impairment (eGFR <30 ml/min) without them knowing they have a health problem. We therefore propose to assess presence of severe renal impairment by a questionnaire rather than by creatinine serum level. Please note that in the main study with gonorrhoea patients, one third of whom are randomized to gentamicin, creatinine will be measured both before and one week after treatment administration.

1. INTRODUCTION AND RATIONALE

With 78 million new cases of gonorrhea in adults aged 15–49 years, gonorrhea is the second most common bacterial sexually transmitted infection (STI) worldwide [1]. Persistent infections may cause severe genital and reproductive tract inflammation and irreversible damage with infertility as a result. Moreover, gonorrhea increases the transmission of human immunodeficiency virus (HIV)[2]. The most recent global report of the World Health Organization (WHO) on antimicrobial resistance (AMR) published in 2014, specifically mentions treatment failures of gonorrhea due to resistance to extended spectrum cephalosporins (ESCs) in 10 countries. ESCs are the treatment of last resort for gonorrhea. Furthermore, decreased susceptibility to ESCs is reported in 36 countries including the Netherlands[3, 4]. It is anticipated to be only a matter of time before gonococci with full resistance to the ESCs spread internationally. Consequently, gonorrhea may become untreatable unless new drugs become available or existing drugs that are presently not used for treatment of gonorrhea are tested for their efficacy in gonorrhea [5-7].

The threat of untreatable gonorrhea is of global concern because there will be a major impact on disease control efforts due to prolonged infections, increased prevalence of serious complications, and also non-urogenital gonococcal diseases such as neonatal infections and disseminated gonococcal infections will become much more common. In addition, untreated gonorrhea is associated with an increased risk of acquisition and transmission of HIV infection[2]. Based on the 2008 global estimates of incident gonococcal infections, the estimate for global disability adjusted life years (DALYs) due to gonorrhea is approximately 440,000[8]. Antimicrobial resistance in gonorrhea will further increase this burden and cost for society. Financial costs for health services and individual patients will increase[9].

This project aims to identify new treatment modalities for uncomplicated gonorrhea using the registered drugs: ertapenem, fosfomycin and gentamicin, which have not yet been proven clinically effective against gonorrhea. Although ertapenem is, like ceftriaxone, a beta-lactam antibiotic, so far Ng strains with resistance to ceftriaxone had much lower minimum inhibitory concentrations (MICs) for ertapenem. For this reason, ertapenem is very likely also an effective antibiotic against ESC-resistant strains. The efficacy of ertapenem in other extended spectrum β-lactamase (ESBL) producing gram-negative bacteria's is previously proven[10-12]. Both fosfomycin and gentamicin have modes of action very different from ceftriaxone, and their efficacy against gonorrhea will be unrelated to the strains' susceptibility for ceftriaxone. Yuan et al. performed a randomized controlled trial (RCT) in which a three-dose regimen of 3 g fosfomycin was compared to dual regimen of ceftriaxone and azithromycin. In this trial, efficacy of both regimens for treatment of uncomplicated gonococcal urethritis in men was shown[13]. However, a non-inferior effectiveness of fosfomycin compared to ceftriaxone plus azithromycin could not be concluded. This study was limited by the small sample size and by the use of different test modalities for diagnosis (gram stain) and test of cure (culture and/or RNA-based Nucleic Acid Amplification Test (NAAT)). Gentamicin has been used for the treatment of gonorrhea in some developing countries for decades [14-16]. However, the effect had never been evaluated in an RCT. A two-arm dual therapeutic regimen trial is ongoing in the UK comparing gentamicin with

ceftriaxone, plus azithromycin in both arms[17]. As mentioned by Kirkcaldy et al, a limitation of an RCT with dual therapeutic arms containing azithromycin is that it is not possible to evaluate the efficacy of the second antibiotic since high-dose azithromycin has demonstrated excellent efficacy against azithromycin susceptible gonorrhea as mono-therapy[18]. Besides, several studies have now conclusively shown that the formerly assumed synergistic effect of a combination of extended spectrum cephalosporin plus azithromycin in fact does not exist [19-23]. We will therefore compare mono-therapeutic regimes since this will allow a true evaluation of the eradicative capacity of each single antibiotic on gonorrhea.

We propose a non-inferiority, double-blind randomized controlled trial to assess whether ertapenem 1000mg, fosfomycin 6g and gentamicin 5mg/kg are good alternatives for the treatment of uncomplicated anogenital gonorrhea compared to the reference treatment in the Netherlands (500 mg ceftriaxone intramuscular (IM)).

It is important to consider that the efficacy of an antimicrobial agent is dependent on the relationship between the MIC for the micro-organism and the exposure of the micro-organism to the agent in the patient. Therefore, we also aim to study the pharmacokinetic and pharmacodynamic properties of gentamicin, ertapenem and ceftriaxone (IM administered) and fosfomycin (orally administered). By combining pharmacokinetic data with antimicrobial susceptibility data (MIC) of circulating *Neisseria Gonorrhoeae* (Ng) strains, Monte Carlo simulations will be performed to predict treatment efficacy under various antimicrobial resistance prevalence conditions, both now and in the near future[24].

The population pharmacokinetics of ceftriaxone, ertapenem and gentamicin have mainly been studied in hospitalized patients that received intravenous administrations of these antibiotics [25-31]. Musson et al.[32], however, studied pharmacokinetic parameters in healthy volunteers after the administration of ertapenem 1000mg intramuscularly without estimating the population pharmacokinetics. For the PKPD analysis of the studied antibiotics, it is important to have an estimation of the population pharmacokinetics after the administration of a single dose of ceftriaxone 500mg IM, ertapenem 1000mg or gentamicin 5mg/kg IM in our substudy population. In the PK substudy, we plan to measure the plasma concentrations within 24 hours after administration of these intramuscular doses, and subsequently estimate the PK on population level by NONMEM analysis.

Bergan et al.[33], and Wenzler et al.[34] studied pharmacokinetics after oral administration of 3g and 4g fosfomycin. Since saturated absorption or other unknown mechanisms might result in a non-linear increase in plasma concentration after administration of an increased dose, we also aim to study the population pharmacokinetics after oral administration of 6g fosfomycin in our population. We do not expect that volunteers and gonorrhea patients differ significantly regarding pharmacokinetic characteristics (renal and liver function, volume of distribution and level of proteins in blood plasma), in contrast to hospitalized patients in whom these characteristics may be strongly affected. Therefore we will study the population pharmacokinetics of ceftriaxone 500mg IM, ertapenem 1000mg IM and fosfomycin 6g orally in volunteers without current gonorrhea or gonorrhea in the preceding two weeks

(n=60), and we will study the population PK of ceftriaxone 500mg IM, ertapenem 1000mg IM and gentamicin 5mg/kg in NABOGO participants (n=60).

As of October 2, 2018 the fosfomycin arm was dropped after the advice of the DSMB board based on an interim analysis performed by an independent statistician. The NABOGO trial is thus being continued as a three-armed RCT. The linking of pharmacokinetic and pharmacodynamic data of fosfomycin with clinical treatment outcomes will enable us to understand the main reasons for treatment failure and will allow us to recommend on future dosing regimens. The simulations based on both population pharmacokinetics and pharmacodynamics will be used to estimate the attainment rate of the pharmacodynamic target of the antibiotic for different concentrations; and eventually we may be able to predict promising dosing regimens.

2. OBJ ECTIVES

Primary objectives

To evaluate whether ertapenem and gentamicin are non-inferior to ceftriaxone in the treatment of uncomplicated anogenital gonorrhea in patients visiting the STI clinic of the Public Health Services in Amsterdam. Treatment effectiveness is determined by the bacterial eradication capacity of the antibiotics, based on a test of cure (TOC) 7-14 days after treatment administration.

Secondary objectives

1. To determine the bacterial eradication capacity of the experimental treatment options (ertapenem and gentamicin) compared to the reference treatment (ceftriaxone) in uncomplicated anogenital gonococcal infections by molecular test of cure after 7-28 days.
2. To determine the bacterial eradication capacity of experimental treatment options compared to the reference treatment at infection sites other than the included infection site (also including pharyngeal gonorrhea) by molecular test of cure after 7-28 days.
3. To determine the type, frequency and severity of adverse events of the experimental treatment options compared to the reference treatment.
4. To determine the time to disappearance of symptoms at the included infection site in the first 14 days after treatment, for the experimental treatment compared to the reference treatment.
5. To determine the clinical and demographic predictors for treatment failure (see chapter 7 for definition).
6
7. To determine the *in vitro* antimicrobial susceptibility (in MIC) of the experimental and reference treatment in all Ng strains collected at all infected anatomical sites of each participant at inclusion and in case of a positive test of cure, and to determine whether a relation exists between MIC and treatment outcome.
8. To determine the population pharmacokinetics of a single dose of ceftriaxone 500mg IM, gentamicin 5mg/kg IM and ertapenem 1000mg IM, and fosfomycin 6g orally administered.

3. STUDY DESIGN

RCT

A double-blind randomized controlled single center non-inferiority trial with two experimental arms and one reference arm. The expected duration of the study is 47 months, of which 27.4 months are needed for recruitment. The study will be conducted at the sexually transmitted infections (STI) outpatient clinic of the Public Health Service in Amsterdam.

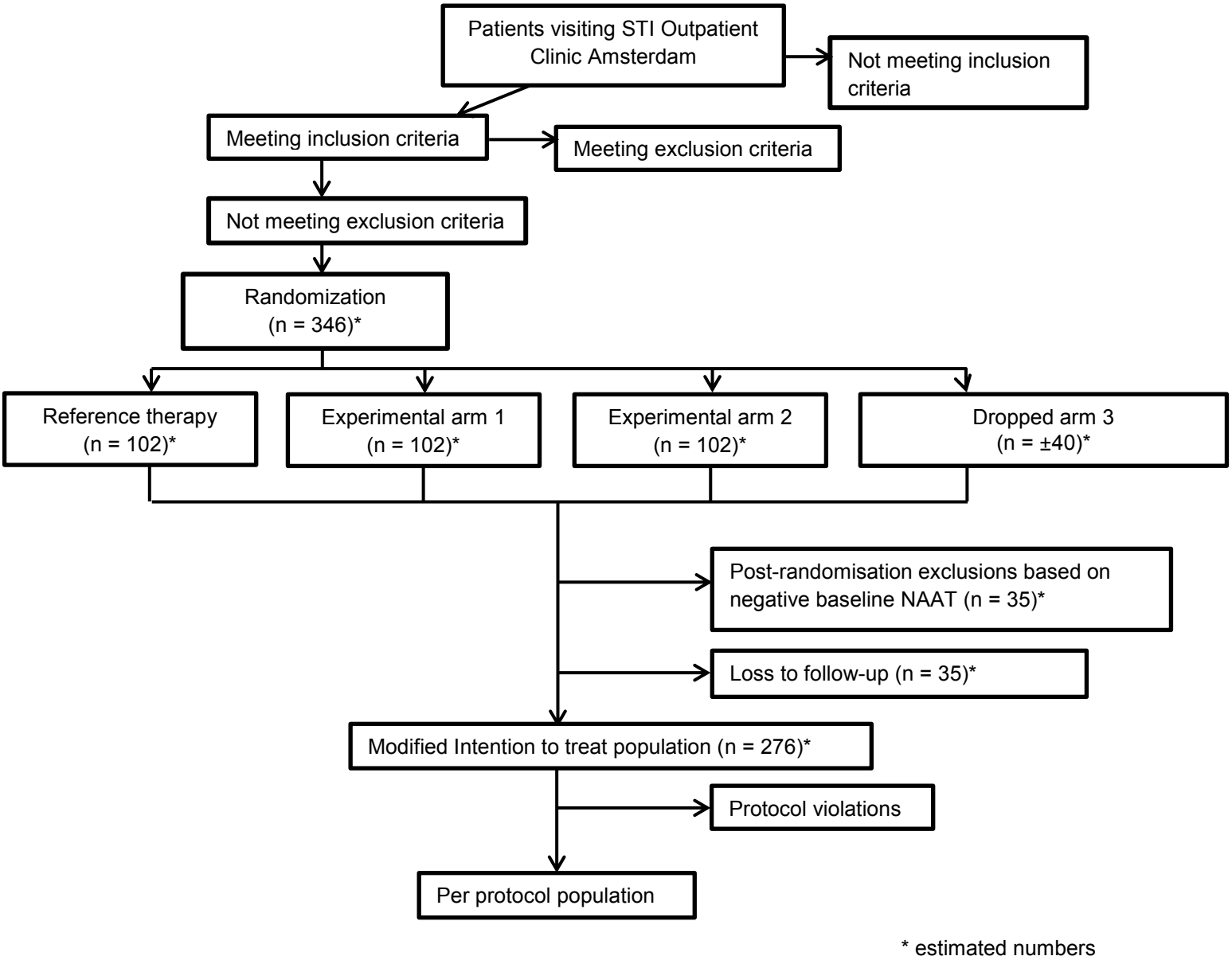


Figure 1. Flow chart of study design and sample size of total recruitment period (18th of September until the inclusion and follow-up of all 346 participants).

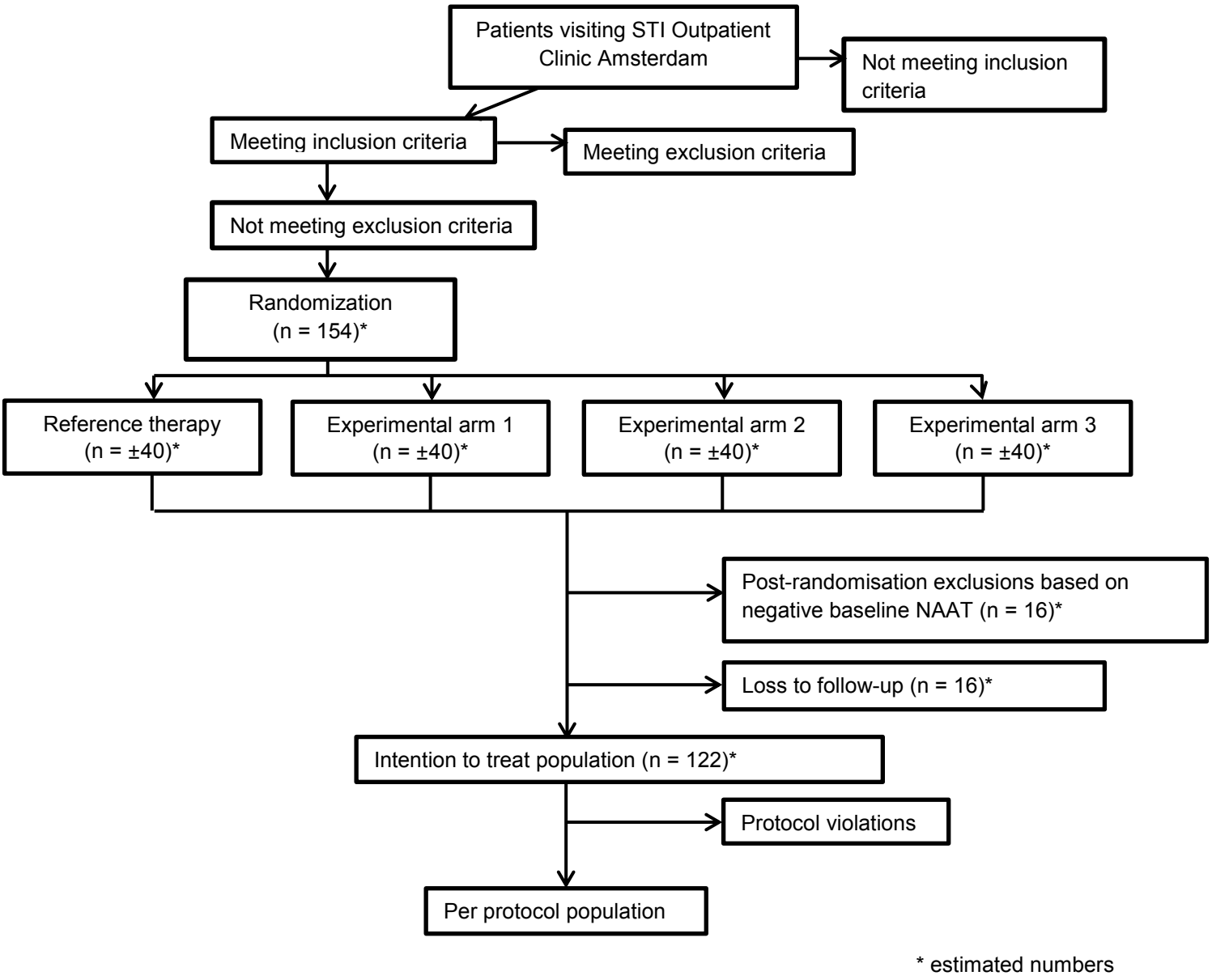


Figure 2. Flow chart of study design and sample size period 1 (from the start of recruitment until the termination of one treatment arm (fosfomycin)).

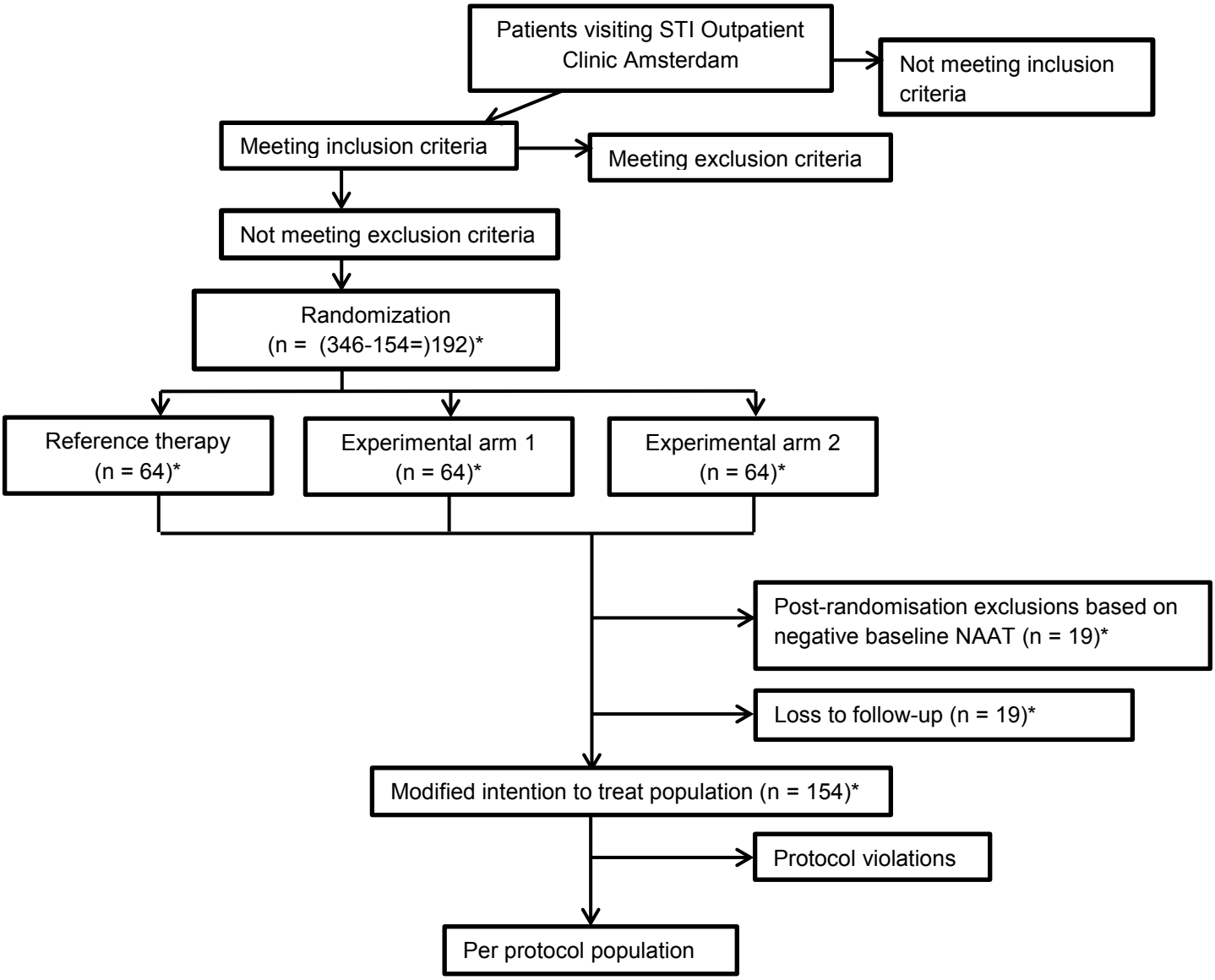


Figure 3. Flow chart of study design and sample size period 2 (from the termination of one treatment arm (fosfomycin) until the total required sample size is reached 1st of October 2018 until 3rd of February 2020)*

PK substudy

To estimate the population pharmacokinetics of ceftriaxone 500mg IM, ertapenem 1000mg IM, fosfomycin 6g PO, and gentamicin 5mg/kg IM we will perform NONMEM based on blood plasma concentrations of these agents within 24hours after treatment administration. We experienced that it is not feasible for NABOGO participants to return 2-3 times within 24hours after treatment administration because they were not prepared for this. We decided to expand the population of the PK substudy with 60 healthy volunteers who did not receive antibiotics in the preceding 2 weeks. From this population we will obtain four blood samples to measure the plasma concentrations for ceftriaxone, ertapenem and fosfomycin at different time points within 24hours after treatment administration. Considering the known side effects of gentamicin (nephro- and ototoxicity) we decided not to expose healthy volunteers to gentamicin. Since gentamicin has a short Tmax, we aim to draw one blood sample in 60 NABOGO participants 30-90 minutes after treatment administration. Following the 1:1:1 randomization scheme, approximately 20 of these participants will have received gentamicin, 20 ertapenem and 20 ceftriaxone. With this we are able to estimate the population Cmax of gentamicin and at the same time compare the first plasma concentration for ceftriaxone and ertapenem in NABOGO participants and healthy volunteers.

We aim to include 120 participants in the PK substudy in total. Of these, 60 participants will be gonorrhea patients and 60 participants will be healthy volunteers recruited at the STI clinic and at universities in Amsterdam.

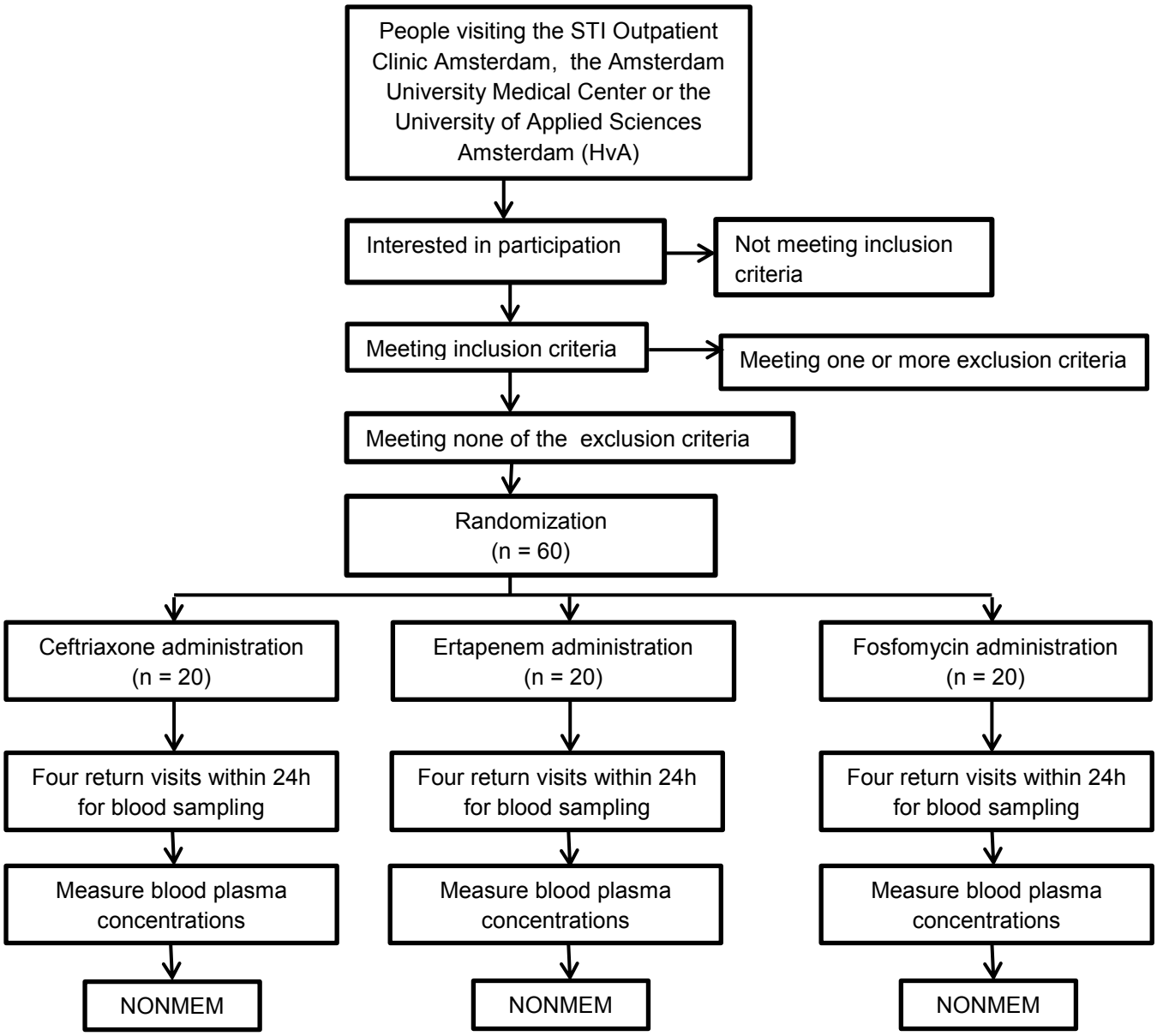
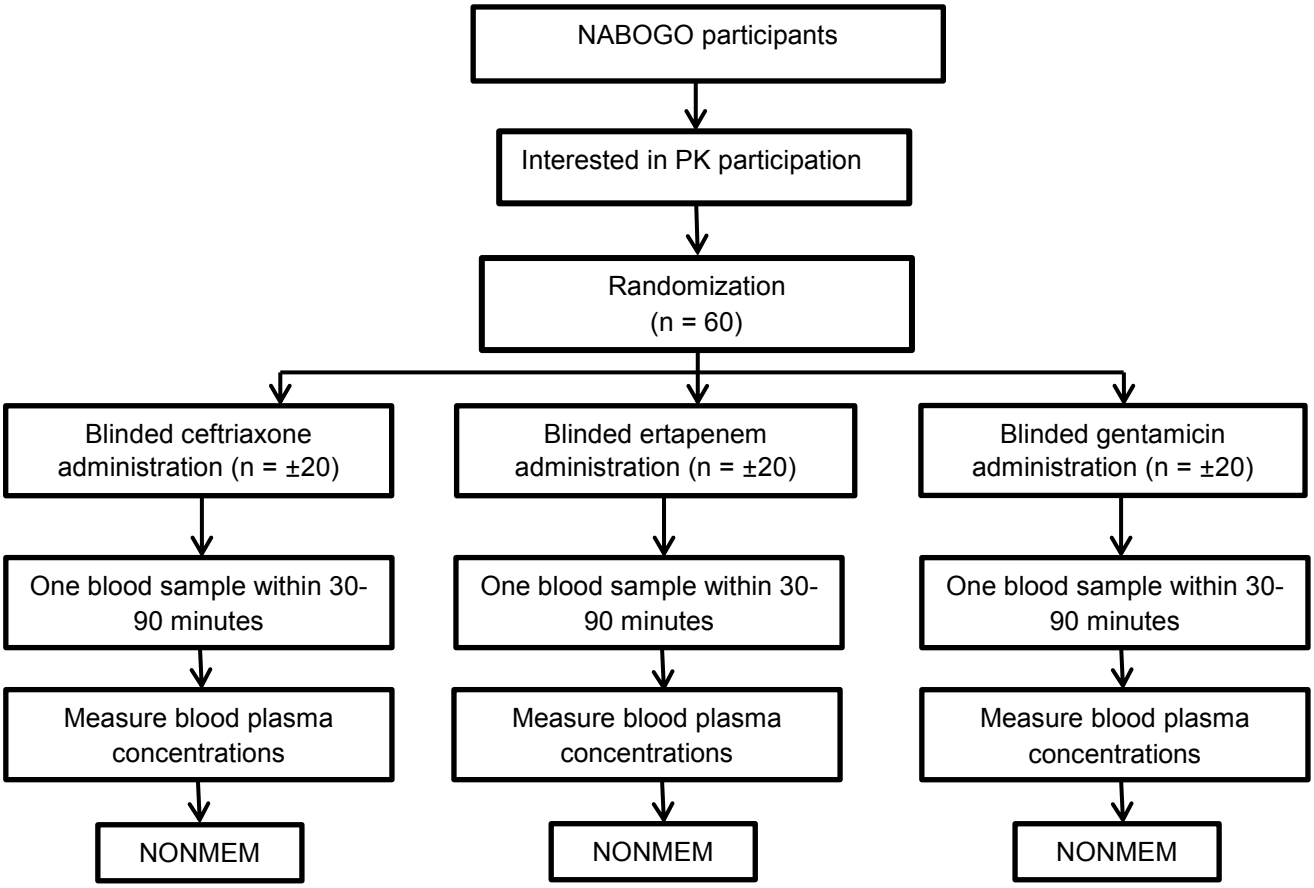


Figure 4. Flow chart of study design and sample size of the PK substudy; healthy volunteers. NONMEM: Non-linear Mixed Effects Modeling.



igure 5. Flow chart of study design and sample size of the PK substudy; gonorrhea patients. NONMEM: Non-linear Mixed Effects Modeling.

4. STUDY POPULATION

4.1.1 Study population main study

Patients visiting the STI Outpatient Clinic Amsterdam and having an uncomplicated anogenital Ng infection can be included in this study. At the STI clinic in Amsterdam we diagnosed 2230 new gonorrhea patients in 2016, or approximately 185 per month. Of these, 2033 patients were male and 197 were female, ages ranged between 15 years and 73 years old with a variety in ethnic backgrounds. Of all gonorrhea cases 28.1% were coinfected with other bacterial STI. Of all anatomical infection sites, in 2016, the anorectal site was most frequently infected (1218 anorectal infections among MSM, 84 anorectal infections among females), followed by the pharyngeal infection site (1026 pharyngeal infections among MSM, 58 pharyngeal infections among females). The urogenital infection was the least infected site (476 urogenital infections among MSM, 144 urogenital infections among heterosexual males, 143 cervical/vaginal infections among females) at the STI clinic in Amsterdam [Annual report STI clinic Public Health Service (GGD) Amsterdam, 2016].

4.1.2 Study population PK-substudy

Attendees of the STI outpatient clinic Amsterdam and students of the Amsterdam University Medical Centre (AUMC) and the University of Applied Sciences Amserdam (HvA) will be approached in order to participate in the PK substudy

4.2.1 Inclusion criteria main study

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- 18 years or older
- Anorectal, cervical/vaginal or urethral Ng infection, diagnosed by the following:
    - Ng-positive Gram-stained smear (intracellular Gram-negative diplococci and leukocytes) and/or
    - Positive for Ng by nucleic acid amplification test (NAAT) (Aptima Combo 2) and/or
    - Positive for Ng by culture
- Provide samples from the included infection site for NAAT and direct culture immediately before treatment
- Willing to abstain from anal, vaginal and oral sex until the test of cure (TOC)-visit, or use condoms during sex
- Willing and able to return for a TOC-visit 7-14 days after treatment
- Provide informed consent
- Accept intramuscular injections

4.2.2 Inclusion criteria PK substudy (healthy volunteers)

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- 18 years or older
- Provide informed consent

4.3.1 Exclusion criteria main study

A potential participant who meets any of the following criteria will be excluded from participation in this study

Pre-randomisation:

- Suspicion of a complicated Ng infection based on signs and/or symptoms indicating pelvic inflammatory disease (PID), epididymitis, prostatitis or gonococcal arthritis*
- Another (sexually transmitted) infection or a suspicion of another infection for which systemic antimicrobial therapy is indicated
- Pregnancy, having a wish to become pregnant or breastfeeding (tested at inclusion visit)
- Not able to read/understand Dutch or English
- HIV infection if:
    - Newly diagnosed HIV infection (upon the inclusion visit) and/or
    - CD4+ cell-count <200 cells/µL (as reported by the patient)
- Known allergy or adverse reactions to ceftriaxone, ertapenemor gentamicin
- Known renal impairment (based on estimated GFR using Cockroft and Gault formula using serum creatinin measured with a point-of-care (POC) test; cut off value renal impairment eGFR ≤ 50 ml/min)
- Known liver cirrhosis (based on history)
- Known congestive heart failure (based on history)
- Known myasthenia gravis
- Known hearing loss or balance disorder, confirmed by an ear-nose-throat (ENT)-doctor or for which an ENT doctor has been consulted and a diagnostic process is still in progress (based on history)
- Concurrent use of any of the following medication:
    - systemic antibacterial antimicrobials other than nitrofurantoin or metronidazole
    - systemic immunosuppressive drugs
    - systemic valproic acid
- Use of any antimicrobial therapy other than nitrofurantoin or metronidazole in the two weeks prior to study enrollment (based on history)
- Previous enrollment in the study
- Concurrent participation in other non-observational medical research*
- Unlikely to adhere to the study protocol

Post-randomisation:

Exclusion of participants from the modified intention to treat analysis (mITT):

- Negative result of Ng NAAT of sample collected on T0 (the day of treatment). This could be the case in the following situations:

1. Negative NAAT in spite of positive gram stain.
2. Positive NAAT on pre-study visit but spontaneous clearance of the infection in the time period between first test and return visit for treatment (=study inclusion visit). A novel sample for NAAT will be collected on the study inclusion visit just before administration of treatment; if these results are Ng-negative a participant will be excluded of mITT.

- Loss to follow-up, i.e. no study visit TOC 7-14 days after treatment administration.

Exclusion from per protocol analysis (PP):

- Exclusion of mITT
- Use of non-study related antibiotics after inclusion and prior to TOC visit
- Condomless sexual contact with the primary anatomical gonorrhea site involved after inclusion and prior to TOC visit
- Other protocol violations

4.3.2 Exclusion criteria PK substudy (healthy volunteers)

A potential participant who meets any of the following criteria will be excluded from participation in this study

Pre-randomisation:

- Pregnancy, having a wish to become pregnant or breastfeeding (tested at inclusion visit)
- Not able to read/understand Dutch or English
- Known allergy or adverse reactions to ceftriaxone, ertapenem, or fosfomycin.
- Known renal impairment (based on history)
- Known liver cirrhosis (based on history)
- Concurrent use of any of the following medication:
    - systemic valproic acid
    - systemic metoclopramide
- Use of any systemic antimicrobial therapy other than nitrofurantoin or metronidazole in the two weeks prior to study enrollment (based on history)
- Concurrent participation in other non-observational medical research (apart from NABOGO RCT)
- Unlikely to adhere to the study protocol

4.4.1 Sample size calculation main study

To calculate the required sample size for this non-inferiority trial we used the R function nBinomial of the “gsDesign“ package. This function computes sample size using the Farrington and Manning method for a trial to test the difference between two binomial event rates [35]. The following parameters were used in the calculation: 98% treatment success in control and experimental arms, 10% non-inferiority δ-margin (a commonly used margin), and 90% power. Three hypothesis tests are considered in which proportion of treatment success are compared between each treatment arm and control. Significance of these tests will be determined using the stepwise Hochberg procedure and power will be based on a one-sided α-level at the first step of this procedure (α=0.025). This results in a sample size of 81 participants per treatment arm. Considering that 20% of participants will be excluded from analysis either due to loss-to-follow-up (10% participants not returning for TOC or exclusion criteria) or *post hoc* exclusion (10% due to false-positive gram stains and self-clearance), we require 102 included participants per treatment arm.

4.2.2 Sample size calculation PK substudy

In order to estimate the population pharmacokinetics, we will measure blood concentration of ceftriaxone, ertapenem and fosfomycin in 20 participants at 4 time points within 24h after administration for each of the three antibiotics, so 60 participants in total [36]. At the same time, we will measure the blood plasma concentration of ceftriaxone, ertapenem and gentamicin in 20 participants at 1 time point within 90 minutes after administration for each of the three antibiotics remaining in the NABOGO trial. With the collected data reliable population pharmacokinetic-pharmacodynamic models will be derived for Monte Carlo simulations.

4.5. Recruitment duration

At the STI clinic in Amsterdam we diagnosed 1667 new anogenital gonorrhea cases in 2016, which is approximately 138 per month. Assuming that 71.9% of them does not have a bacterial co-infection and will be eligible to participate in this trial and assuming that 30% of invited patients consent to participate and does not meet exclusion criteria, we may expect 30 inclusions per month. To allow for a slow inclusion of 25 per month, we will need a recruitment period of 408/25 = 16.3 months.

The recruitment rate in the first 11 months of the trial was 2.8 participants per week. Since we need 102 participants in each of the remaining three treatment arms (resulting in a total sample size of ±346 participants), we expect that we need 124 weeks in total. Recruitment started on 18th of September 2017 and is expected to be completed on 3 of February 2020.

4.6 Patient registration

Inclusion of participants will be performed by research nurses and the research coordinator. After obtaining written informed consent, the research coordinator will keep the informed consent form. A study number will be generated at inclusion and stored on a coding list. All participants receive a ‘study identification card’ with contact information of the study team and a personal study number of

which should be kept by the participant. This ‘study identification card’ does not contain any personal information of the participant.

5. TREATMENT OF SUBJECTS

5.1 Investigational treatment

Participants will be treated with one of the two experimental therapies (intramuscular gentamicin and ertapenem) or the reference therapy (intramuscular ceftriaxone). Participants and the treating health professional will be blinded for the treatment allocation. For blinding purposes, all participants receive two intramuscular injections (gentamicin will be administered in two intramuscular injections, when allocated to the ertapenem or ceftriaxone group participants will be given one intervention intramuscular injection and one placebo intramuscular injection).

5.2 Use of co-intervention

There will be no use of co-intervention. Nevertheless participants are asked to refrain from sexual intercourse or use condoms between treatment and follow-up visit in order to minimize the occurrence of re-infection.

5.3 Escape medication

In case of treatment failure (see chapter 8.1.1 for definition), patients will be treated with the current reference therapy in an increased dose, ceftriaxone 1000mg intramuscular, plus azithromycin 1000mg orally. Ceftriaxone is currently still 100% effective in the Netherlands. However, it is possible that treatment failure occurs even after receiving ceftriaxone 500mg (as study therapy). Since Ng cultures are not always successful (positivity rate is approximately 80% in our laboratory) and thus we might not always have MIC values of the Ng strains, we choose to double the dose of ceftriaxone and to add azithromycin to the escape medication regime. Azithromycin is effective in the majority of Ng strains. If the Ng bacteria have been cultured from a case of treatment failure either at the day of inclusion and/or follow-up visit, and we know that the Ng strain is resistant to azithromycin and susceptible to ceftriaxone, the participant will receive ceftriaxone 1000mg only.

6. INVESTIGATIONAL PRODUCT

6.1 Names and description of investigational medicinal products

There will be three arms consisting of experimental treatments and one control arm consisting of the current reference treatment.

- Reference treatment: single dose ceftriaxone 500 mg IM, dissolved in 2 ml lidocaine 1% supplemented with 0.9% NaCl until 10 ml (2 x 5 ml).
- Experimental arm 1: single dose ertapenem 1000 mg IM, dissolved in 3.2 ml lidocaine 1% supplemented with 0.9% NaCl until 10 ml (2 x 5 ml).
- Experimental arm 2: gentamicin 5 mg/kg IM injection once (solution 40 mg/ml), if indicated supplemented with 0.9% NaCl until 10 ml (2 x 5 ml). Maximum gentamicin dose is 400 mg because this is diluted in 10ml which is the maximum volume for 2 intramuscular injections.
- Experimental arm 3: fosfomycin 6 gram trometamol orally once (PK substudy only)
- Placebo intramuscular injection: 5 ml or 10 ml (2 x 5 ml) 0.9% NaCl (when receiving oral therapy 10 ml 0.9% NaCl will be administered, and when receiving ertapenem or ceftriaxone 5 ml 0.9% NaCl will be administered).

6.2 Summary of findings from non-clinical studies

See summary of product characteristics.

6.3 Summary of findings from clinical studies

See summary of product characteristics.

6.4 Summary of known and potential risks and benefits

Ceftriaxone

Ceftriaxone is the standard therapy for Ng infections in the Netherlands. There is no resistance among Ng strains to ESCs reported in the Netherlands and ceftriaxone is well tolerated. Thus, in this trial ceftriaxone will not cause any additional risks in comparison to the general population with gonorrhea. Consequently there is no need for extra measures to reduce expected risks.

Ertapenem

Ertapenem is safely used for several indications worldwide both in intramuscular and intravenous form (for more detailed information see chapter 12.1) [37]. In Europe, ertapenem is not yet registered as intramuscular injection, however it is safely used intravenously [38]. We do not expect any harm from a single intramuscular dose in our young and relative healthy population. Because of the influence of ertapenem on the valproic acid plasma levels, patients using valproic acid are excluded from this trial.

Fosfomycin

Fosfomycin is safely used for several indications worldwide. The usual dose of oral fosfomycin in urinary tract infections is a single dose of 3g. Based on Monte Carlo simulations to predict effective

treatment dosage, 6g is expected to be effective in gonococcal infections. Since intravenous doses are administered up to 24g in 2-3 doses per day, we do not expect toxicity from a 6g oral dose. A few clinical studies have been performed on (either intramuscular or oral) fosfomycin in patients with uncomplicated gonococcal urethritis, none of these studies reported any severe events [14, 30, 31]. Participants of this trial are assured not to take metoclopramide in the first 7 days. In case of the intake of metoclopramide on the day of inclusion or in case of the intention to take metoclopramide in the following days, patients are excluded from this study.

Gentamicin
Since gentamicin is widely used for different indications and earlier research on a single dose intramuscular injection of 240-280mg [18, 39-42] in patients with gonorrhea did not show any severe side effects, the hypothesis is that it is an efficient and safe product for this indication. There is limited knowledge on the prevalence of nephro- and ototoxicity as a result of a single dose gentamicin [43]. For this reason, renal function will be measured before treatment administration and people with an impaired renal function will be excluded from this study. Additionally, at the follow-up visit 7-14 days after treatment, renal function will again be tested in order to follow the effects of a single dose gentamicin on renal function. Also hearing problems and balance disorder will be evaluated before and after treatment.

6.5 Description and justification of route of administration and dosage

Ceftriaxone, ertapenem and gentamicin will be administered intramuscularly, as oral administration is not possible. Furthermore, intravenous administration is less comfortable for patients, not necessary and not possible at the STI outpatient clinic. In Europe, ertapenem is only registered for intravenous administration. In the United States of America (USA) ertapenem is approved by Food and Drug Administration (FDA) both for intravenous and intramuscular administration for various indications [37].
For blinding purposes all participants receive two intramuscular injections.

6.6 Dosages, dosage modifications and method of administration
Dosages for ertapenem, fosfomycin and gentamicin are based on Monte Carlo simulations using pharmacokinetic information from literature [44-56], and MIC data of Ng strains isolated in Amsterdam
Dosage of ceftriaxone is according to current Dutch STI guidelines [57].
Monte Carlo simulations of 5 mg/kg gentamicin (max 400 mg) IM demonstrated a median maximal concentration (Cmax) of 16 mg/L (95% CI: 8-22 mg/L (see figure 2). With a MIC of 1 the ratio Cmax/MIC is well above 10 which is considered a PD target for gentamicin.

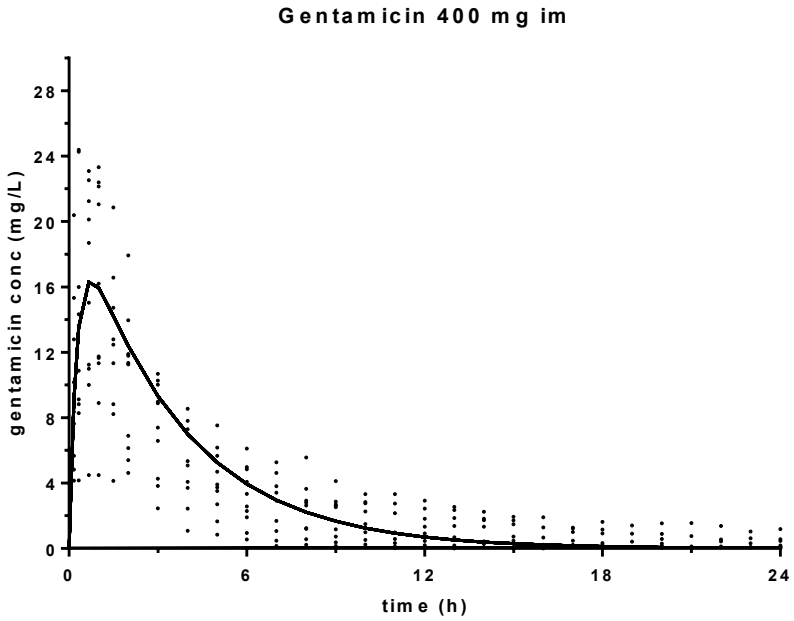


Figure 6: Monte Carlo simulation of 5 mg/kg (max 400 mg) gentamicin IM

Monte Carlo simulations of 1000 mg ertapenem IM resulted in unbound concentrations 9.6 hours above MIC in 100% of the patients with MIC up to 0.25 mg/L (Figure 3). This is well above the MIC90 observed in our outpatient clinic (0.016 mg/L).

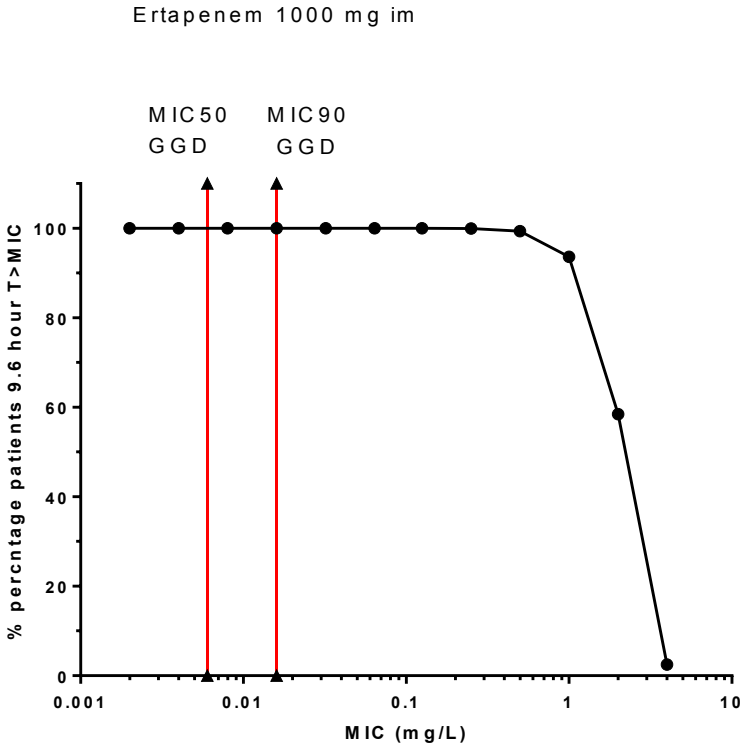


Figure 7: Monte Carlo simulation of 1000 mg ertapenem IM

Monte Carlo simulations of 6 gram oral fosfomycin resulted in concentrations above an MIC of 10 mg/L during an 8-hour period for 95% of the patients. Lopez Gracia reported a similar exposure after a single IM dose of 4 gram; in this study this dose produced 100% eradication [30].

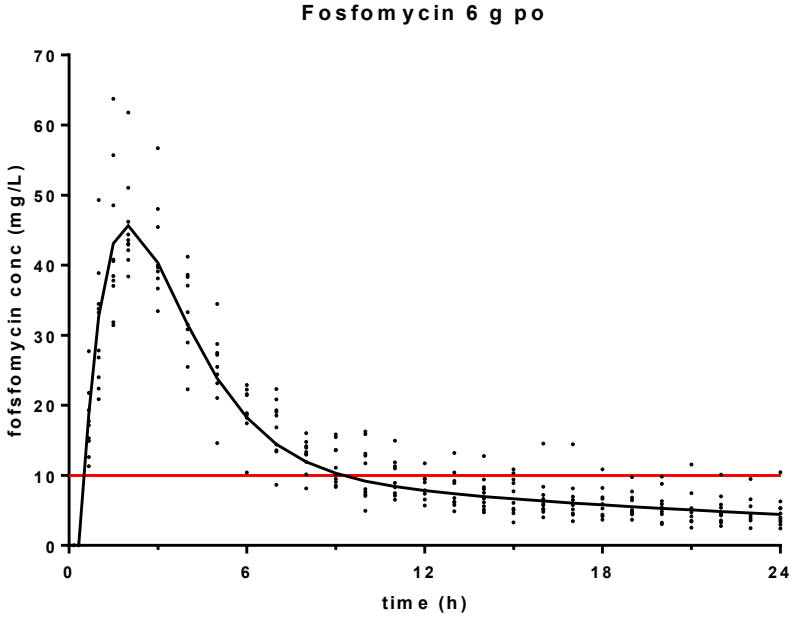


Figure 8: Monte Carlo simulation of 6 gram oral fosfomycin

6.7 Preparation and labeling of Investigational Medicinal Product

This study will be performed in a double-blind fashion. The patient, study nurse and the study coordinator will be blinded to treatment allocation.

The medication will be prepared by an independent medical doctor or nurse from the STI clinic. When a patient is included in the study, this independent medical person performs the randomization and prepares the medication according to the allocated treatment arm. Subsequently, the blinded medication is delivered to the study coordinator or study nurse, who will administer the blinded medication to the participant. A list will be kept by the independent medical person with the participant study ID linked to the allocated study arm. Four to five medical doctors and nurses from the STI clinic will be trained to function as an independent treatment preparer.

6.8 Drug accountability

Ceftriaxone and gentamicin are registered at the European Medicines Agency (EMA) in this form and mode of administration and therefore will be available from any pharmacy. However, as stated before, ertapenem is only registered at the EMA for IV administration. The IV formulation is used for IM administration in the USA and this method is FDA approved for several indications. We will use the IV formulation likewise for IM administration. Batch numbers of medication used will be recorded.

For intramuscular placebo we will use 0.9% NaCl.

7. METHODS

7.1 Study endpoints

7.1.1 Primary endpoint

Bacterial eradication of Ng infection at the included (anal or urogenital) infection site, based on a test of cure (TOC) using an RNA-based NAAT (Aptima Combo 2 assay) at the follow-up visit 7-14 days after treatment (T7). Subsequently, the difference in treatment success rates between each investigational treatment and the reference treatment will be measured.

Treatment success of anal, urethral, vaginal and pharyngeal infection sites is defined as a negative test of cure.

Treatment failure of the anal, urethral and vaginal infection sites is defined as 1) a positive test of cure 7-14 days after treatment, or 2) a positive test of cure less than 7 days after treatment in combination with persisting symptoms and/or a positive gram stain 3-6 days after treatment.

Treatment failure of the pharyngeal infection site is defined as 1) a positive NAAT ≥ 14 days after treatment, or 2) a positive Ng culture ≥ 7 days after treatment.

7.1.2. Secondary endpoints

1. Bacterial eradication of Ng infection at the included (anal or urogenital) infection site, based on TOC using an RNA-based NAAT 7-28 days after treatment.
2. Bacterial eradication of Ng infection at any infected site(s) (anal, urogenital and/or pharyngeal) not included in the primary endpoint analysis, based on a TOC using an RNA-based NAAT 7-28 days after treatment.
3. Any adverse events (type, frequency and severity) occurring during 28 days following the start of treatment.
4. *In vitro* MIC of all Ng strains for all study antimicrobials, determined by e-test on culture at inclusion (before treatment, T0) and at the TOC follow-up visit (T7).
5. Clinical cure:
    a. Symptoms of Ng infection (such as pain, irritation/itch, redness, any discharge, bleeding, changed defecation pattern and/or swelling) from treatment to TOC visit (7-14 days).
    b. Time (in days) from the start of treatment to disappearance of symptoms of Ng infection.
    c. Signs of Ng infection (such as mucosal fragility, redness, discharge, bleeding and/or swelling) assessed at physical examination if indicated based on symptoms, at T0 and T7.
6. Pharmacokinetic characteristics of study drugs in peripheral blood up to 24 hours after administration.

7.2 Randomization, blinding and treatment allocation

7.2.1 Randomization

Participants will be randomized in a 1:1:1 ratio to the three treatment arms. Randomization will be performed using random permuted blocks assuring equal distribution to all treatment arms. The length of the blocks will be random to avoid any selection bias (3-9 participants per block). A computer randomization program ALEA, which is validated for use in GCP trials and will be provided by the AMC Clinical Research Unit, will be used to perform allocation.

7.2.2 Blinding

This study will be performed in a double-blind fashion. The patient, study nurse, the study coordinator and principal investigator will be blinded to treatment allocation.

There will be an independent medication preparer available for randomization, treatment allocation, preparation and blinding of medication. When a patient is included in the study, the medication preparer performs the randomization and prepares the medication according to the allocated treatment arm. The independent medication preparer will deliver the blinded medication to the study coordinator or study nurse, who subsequently will administer the medication to the participant. A list will be kept by the independent medication preparer with the participant study ID linked to the allocated study arm. Medical doctors and nurses from the STI clinic will be trained to function as an independent treatment preparer.

7.2.3 Treatment administration

All participants receive two intramuscular injections of 5 ml in the upper outer quadrant of each gluteus maximus muscle.

7.2.4 Indication for breaking the randomization code

An AE that needs further treatment by a medical doctor could be an indication for breaking the randomization code in a particular participant. The appearance of a SAE that is determined to be related to the study treatment could also be an indication for deblinding the treatment allocation in a particular participant.

7.3 Study procedures

All participants will receive standard care including counseling, partner notification, and diagnostics for other STIs if indicated.

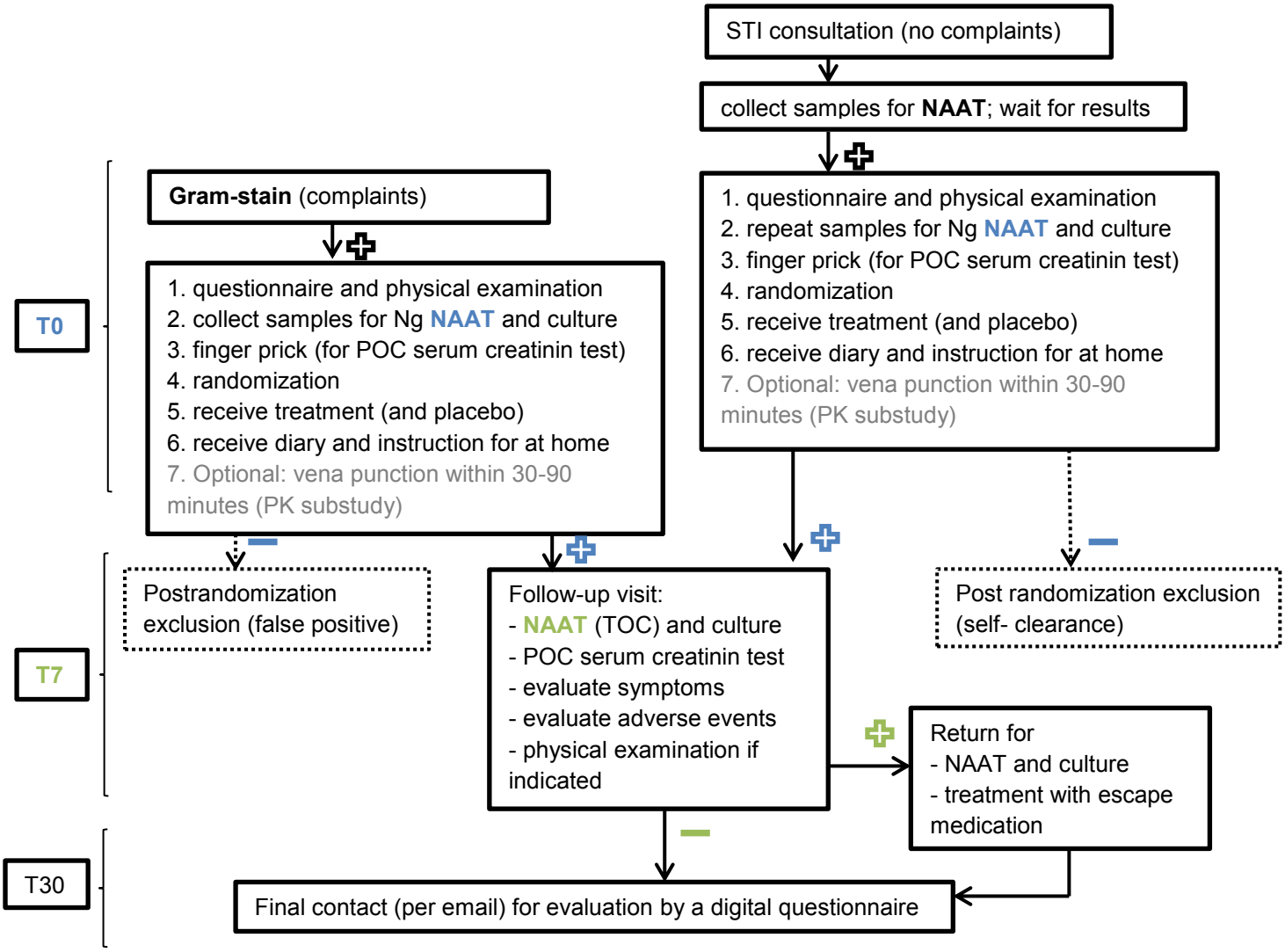


Figure 9. Flowchart of study procedures

Persistence or worsening of symptoms after treatment

Participants who contact the STI clinic in less than 7 days after treatment administration because of persistence or worsening of symptoms, will be evaluated by the study coordinator or the principal investigator. If indicated, a Gram stain will be repeated. If the Gram stain is negative, a watchful waiting policy will be followed regarding the Ng infection and the participant will be advised to come back at the follow-up visit 7 days after treatment administration. Further diagnostics can be initiated for other (suspected) diseases. When the repeated Gram stain is positive >48 h after treatment administration, samples will be collected for NAAT and culture, and the study physician decides whether to treat with escape medication or not, depending on the severity of complaints. Ng RNA may still be detectable within 7 days after treatment, even when the infection has been effectively treated [58]. This means that a positive NAAT within 7 days is not a reliable indicator for treatment failure. If escape medication is given and the NAAT is positive, this case will be considered as treatment failure in the intention to treat (mITT), but will be excluded from the per protocol analysis. When escape

medication is given and the culture is positive, this case will be treated as a treatment failure in both the mITT- and per protocol (PP) analysis.

Questionnaire

The following information will be obtained by a questionnaire at the day of inclusion and at the follow-up visit at day 7-14: symptoms (pain, irritation/itch, redness, discharge, bleeding, and/or swelling, duration of symptoms), sexual behavior, and at the follow-up visit and by an e-questionnaire at day 30 (a link to a secured digital questionnaire will be sent by email): any adverse events (in particular rash and hearing problems, vestibular problems or tinnitus). The e-questionnaire at day 30 will also contain an evaluation of the experiences of participating in this trial.

Diary

All participants are requested to keep a diary to record the presence of complaints (either symptoms and adverse events), sexual activity and medication use.

Physical examination

Signs (mucosal fragility, redness, discharge, bleeding and/or swelling) at physical examination of the included infection site are scored by a study nurse, study physician or clinician during clinic visits at the day of inclusion (T0) and at the follow-up visit for TOC (T7) if indicated based on symptoms.

Laboratory tests

- <u>Ng RNA-based NAAT (Aptima Combo 2 assay)</u>

  The RNA-based NAAT is the test of first choice for test of cure because of its high specificity and superior sensitivity compared to bacterial culture[59]. It has been recently recommended to perform the test no sooner than 7 days after treatment [58].
  - Men who have sex with men (MSM): anal and pharyngeal swabs and urine will be obtained at T0 and at T7, test of cure (TOC).
  - Females: anal, pharyngeal and vaginal swabs will be obtained at T0 and T7 (TOC)
  - Heterosexual men: urine will be obtained at T0 and T7 (TOC).

  In case of treatment failure another NAAT of the anatomical location(s) that were infected at T0 will be performed just before the administration of escape medication and 7-14 days later (a second TOC).

- <u>Ng culture</u>
  - MSM: anal, pharyngeal and urethral swabs will be obtained at T0 and at T7 (TOC).
  - Females: anal, pharyngeal and vaginal swabs will be obtained at T0 and at T7 (TOC).
  - Heterosexual men: urethral swabs will be obtained at T0 and at T7 (TOC).

  In case of treatment failure additional cultures of the anatomical locations that were infected at T0 will be performed just before the administration of escape medication and 7-14 days later (a second TOC).

- Molecular typing
  In case of a Ng-positive culture at T0 and T7, strain identification of both the Ng strains will be performed by genotyping in order to identify whether these strains are genetically identical.

- Minimal inhibitory concentrations
  MIC values of ceftriaxone, ertapenem, fosfomycin and gentamicin for all Ng strains by Etest. EUCAST certified breakpoints of MIC values for *Neisseria gonorrhoeae* do not exist for ertapenem, fosfomycin and gentamicin. For this reason, it is not possible to draw any conclusions on susceptibility/resistance before the analysis.

- Creatinine and calculation of the estimated glomerular filtration rate (eGFR)
  Finger prick to collect a drop of blood (32 µL) for a point-of-care serum creatinin test at T0 and at T7 will be performed in order to test for renal impairment (an exclusion criterion and possible adverse event of gentamicin). Renal impairment is defined by a eGFR ≤50 ml/min (calculated with Cockroft-Gault).

- Plasma concentration of antibiotics and albumin
  In participants of the substudy, a venapunction will be performed to collect 4,5 ml blood at four time points within 24 hours after treatment administration . In these blood samples the plasma concentration of the antibiotics and albumin (only in the first sample) will be measured. The maximal volume is 18 ml in total.

Re-infections: to minimize the risk of re-infections
All participants are requested to abstain from sexual intercourse until their TOC visit, or consistently use condoms, and report their behavior in a diary.

Storage of bodily material
According to routine procedures at the laboratory body material with negative test results will be destroyed after 2 weeks. Bodily material of positive test results will be stored until 5 years after the end of the trial, provided that consent is obtained.

PK substudy

1. Gonorrhea patients (also participants in main study)
   Each participant will be requested to participate in the PK substudy as well. If participants agree to provide one extra blood sample, a vena punction will be performed and maximum 4.5mL blood will be drawn 30-90 minutes after the blinded treatment (ceftriaxone, ertapenem or gentamicin) administration.
2. Healthy volunteers
   For this substudy participants will receive one of three antibiotics (ceftriaxone 500mg IM, ertapenem 1000mg IM, or fosfomycin 6g orally) at a pre-arranged moment and will return four

times within 24 hours in order to obtain one blood sample each timepoint. In total 18 ml blood will be obtained by venapuncture to measure the plasma concentration of the antibiotic, creatinine, ASAT, ALAT and albumin levels (the latter 4 values will only be measured in one blood sample).

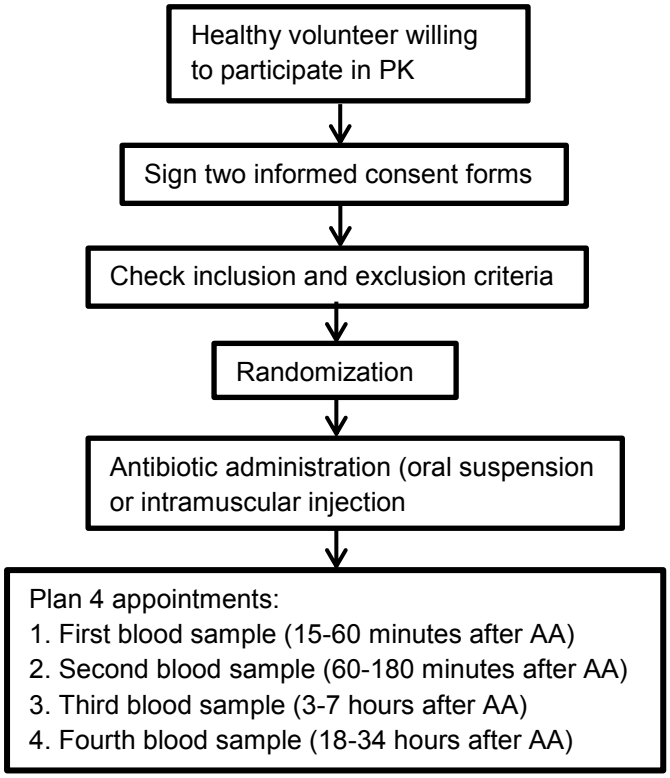


Figure 10. Flowchart of the PK substudy for healthy volunteers

7.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason without any consequences.

7.5 Replacement of individual subjects after withdrawal

This longitudinal study has a relative short follow-up period. If patients are withdrawn from the study when recruitment is still ongoing, we will replace them by new inclusions until at least 346 patients are included.

7.6 Follow-up of subjects withdrawn for treatment

If subjects are withdrawn from the study, follow-up is still recommended in order to evaluate adverse events. Furthermore, patients will still be able to routinely visit the STI clinic.

7.7 Premature termination of study

Based on the scheduled interim analysis after the inclusion of one third of the total required sample size of the four-armed trial, the DSMB advised to discontinue with one treatment arm (fosfomycin). We thus continue this RCT with three arms (ceftriaxone, ertapenem and gentamicin), Other reasons

to stop a treatment arm would be situations in which any patient dies, any patient requires emergency surgery, or any patient suffers a permanent and irreversible disability, and it can be shown that this event was related to participation in this trial.

8. SAFETY REPORTING

8.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardize participant health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all participants are kept informed.

8.2 AEs, SAEs and SUSARs

8.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a participant during the study, whether or not considered related to the trial procedure or the experimental intervention. All adverse events reported by the subject or observed by the investigators, meeting all of the following criteria will be recorded:

- Severity: grade 3 and 4, or grade 1 and 2 lasting longer than 1 week, graded by the CTCAE (Common Terminology Criteria for Adverse Events, v4.03, June 2010).
- Causality: there needs to be a reasonable suspicion of the AE being an effect of the medical treatment of uncomplicated anogenital gonorrhea.
- Time window: between the moment of inclusion and the day 30 follow-up visit.

8.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that results in death or;

- Is life threatening (at the time of the event);
- Requires hospitalization or prolongation of existing inpatients' hospitalization;
- Results in persistent or significant disability or incapacity;
- Any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been, based upon appropriate judgment by the investigator.

An elective hospital admission will not be considered as a serious adverse event.

Medically significant serious adverse events considered related to the investigational product by the investigator will be reported through the web portal ToetsingOnline to the accredited METC that approved the protocol within 7 days of first knowledge. Line-listings of all other SAEs will be reported in the annual safety report.

8.2.3 Suspected unexpected serious adverse reactions

Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered.

Unexpected adverse reactions are SUSARs if the following three conditions are met:

1. the event must be serious (see chapter 9.2.2);
2. there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
3. the adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in:
   - Summary of Product Characteristics (SPC) for an authorised medicinal product;
   - Investigator's Brochure for an unauthorised medicinal product.

The sponsor will report expedited the following SUSARs through the web portal *ToetsingOnline* to the METC:

- SUSARs that have arisen in the clinical trial that was assessed by the METC;
- SUSARs that have arisen in other clinical trials of the same sponsor and with the same medicinal product, and that could have consequences for the safety of the subjects involved in the clinical trial that was assessed by the METC.

The remaining SUSARs are recorded in an overview list (line-listing) that will be submitted once every half year to the METC. This line-listing provides an overview of all SUSARs from the study medicine, accompanied by a brief report highlighting the main points of concern.

The expedited reporting of SUSARs through the web portal ToetsingOnline is sufficient as notification to the competent authority.

The expedited reporting will occur not later than 15 days after the sponsor has first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.

8.3. Annual Safety report

In addition to the expedited reporting of SUSARs, the sponsor will submit, once a year throughout the clinical trial, a safety report to the accredited METC, competent authority, and competent authorities of the concerned Member States.

This safety report consists of

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;
- a report concerning the safety of the subjects, consisting of a safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation;
- a list of all medically non-significant serious adverse events and all serious adverse events considered unrelated to the investigational product.

8.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow-up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist may be required.

8.5 Data safety monitoring board (DSMB)

We expect the additional risks of participating in this study to be low-moderate. In case of moderate extra risks the use of a Data Safety Monitoring Board (DSMB) could be determined per study. In this study a DSMB will be installed to timely detect possible inefficacy of one or more experimental treatment arms. The included antimicrobials are widely used for other indications and there is ample experience with these drugs suggesting minimal safety risks. Nevertheless, if a SAE or important AE occurs, the investigators first evaluate whether a relation to the study medication is probable and whether deblinding is indicated. Subsequently, the investigators propose their plan to the DSMB who will eventually recommend on how to proceed. Detailed information on the DSMB procedures can be found in the DSMB-charter (K5).

9. STATISTICAL ANALYSIS

9.1 Primary study parameters

We will conduct two analyses on differently defined study populations.

(1) modified ITT (mITT) analysis will include all patients randomized, apart from pre-specified exclusions (patients based on a microscopical gram result indicating gonorrhoea, but in whom gonorrhoea was not confirmed by NAAT or culture; and patients with confirmed gonorrhoea, but in whom clearance of Ng prior to administering study treatment was confirmed by a negative NAAT at T0). In all mITT analyses, participants will be considered to have failed treatment if:
- they were given escape medication within 7 days after treatment administration*;
- if the result of the TOC NAAT between day 7 and 14 is positive

* Exception: participants who were given escape medication within 7 days after treatment, but whose NAAT test result after initial study treatment was negative prior to escape medication will be regarded as treatment success.

Participants are considered lost to follow-up in the analysis if there is no outcome assessment 7-14 days post treatment. These participants will not be considered in the primary modified intention to treat analysis. To test the robustness of the analysis, we will conduct two sensitivity analyses as follows: i) considering the LTFU participants as treatment failures and ii) considering the LTFU participants as treatment successes.

(2) PP analysis will only include patients who:
- were considered in mITT analysis;
- completed treatment;
- did not receive unpermitted medication between time of treatment and time of outcome assessment;

- did not report condomless sex with the primary anatomical gonorrhea site involved
- had an outcome assessment between 7 and 14 days post-treatment;
- had no protocol violations.

If a single participant is infected with *Neisseria gonorrhoeae* at more than one anatomical site, only one site will be included in primary analysis. Because the prevalence of cervical infections is much lower than urethral or rectal infections, cervical infections will preferentially be included over rectal infections in the same patient. The order of inclusion will be: 1) cervical infections, 2) urethral infections and 3) anal infections.

The difference in treatment success rates between each of the experimental treatments and the reference treatment will be calculated. The 97.5% confidence interval around this risk difference will be calculated using the R function ci.pd of the R “Epi” package. This package uses the Newcombe method to estimate the confidence intervals for the difference between independent proportions [60]. If the lower limit of the confidence interval around the risk difference between an experimental treatment and the control treatment does not exceed -10% (the predefined non-inferiority margin), the

experimental treatment will be declared non-inferior to the reference treatment. *P*-values will be estimated by the R "gsDesign" package. The Hochberg stepwise procedure will be used for three hypothesis tests. *P*-values for the three tests will be placed in order from lowest to highest. Sequential thresholds of one-tailed Type 1 error are applied to each respective test to determine significance: 0.025, 0.0125 and 0.0083.

We will perform a sensitivity analysis in which participants with a positive TOC based on a re-infection (proven by non-identical genetic type determined by genotyping) will be coded as treatment success instead of treatment failure.

9.2 Secondary study parameters

Secondary outcomes (eradication capacity of study drugs up to 28 days and eradication capacity of study drugs at different infection sites not included in primary objective) will be analyzed using equivalent methods as above.

Analysis of adverse events to a particular medication will include all participants who received the medication. Adverse event analysis will be descriptive. Frequency counts and percentages of the prespecified main categories of AEs will be presented by treatment arm.

In order to assess whether there are any differences in distributions of baseline covariates across treatment arms, demographic and clinical data at baseline will be compared between the four treatment groups.

We will perform explorative analyses to assess determinants of treatment failure using univariable and multivariable logistic regression. Covariables to include in analysis are as follows: previous episodes of gonorrhea, HIV status, presence and duration of symptoms, age, gender and ethnicity.

It is possible that non-inferiority for one or more treatments is not met in a specific patient stratum. Non-inferiority across sub-groups will be evaluated using a test for interaction developed by Wiens and Heyse [61].

The population pharmacokinetics will be estimated for each study antibiotic based on the measured plasma antibiotic concentrations using non linear mixed effects modeling (NONMEM). The estimated population pharmacokinetic and antimicrobial susceptibility data of circulating Ng strains will be combined, and Monte Carlo simulations will be performed in order to predict treatment efficacy under various antimicrobial resistance prevalence conditions.

9.3 Interim analysis

A single planned interim analysis was performed by an independent statistician who informed the Data Safety Monitoring Board (DSMB) in order to timely reduce the risk of participants being exposed to a significantly less effective antimicrobial agent. After including one third of the total intended sample size, the proportion of treatment failures was calculated per treatment arm. The predefined stopping rule was: 40% or more of the participants in a given arm are not successfully treated (i.e. less than 60% have a negative TOC). The antimicrobial agent associated with this arm is considered

ineffective and the study arm will be discontinued. The steering committee considered this proportion of treatment failure as clinically unacceptable. When assuming a cure rate of 98% in the reference treatment, we calculated that with a number of 33 participants per treatment arm (approximately one-third of the final number of participants per treatment arm), the 97.5% confidence interval (using the Newcombe method) around the risk difference between the experimental treatment (cure rate 60%) and the reference treatment (cure rate 98%) will be -56% to -14%. This would mean that the experimental treatment is significantly worse than the reference treatment with the upper limit under the non-inferiority margin of -10%.

After discontinuation of the fosfomycin treatment arm, we will continue the blinding for the participants that were already included in the study before the termination of this/these arm(s). However, we deem it unethical to continue unnecessary exposure of participants to study procedures exclusively related to the terminated treatment arm. Therefore, we reveal to study staff and future participants which treatment armis terminated. This will allow us to adapt the protocol for the remaining treatment arms and therefore avoid continued unnecessary exposure of participants to procedures related to the terminated study arm.

The outcome of the planned interim analysis resulted in the advice of the DSMB to discontinue the fosfomycin arm of this trial. Therefore we decided to stop the administration of fosfomycin 6g orally to NABOGO participants on October 2, 2018; also the oral placebo was no longer administered.

If required, the DSMB will analyze safety of participants on an ad hoc basis and guide recommendation for continuation of the study or early termination because of clear harm to the participant. We will stop the study early if any patient dies, if any patient requires emergency surgery, or if any patient suffers a permanent and irreversible disability, and it can be shown that this event was related to participation in the clinical trial.

10. ETHICAL CONSIDERATIONS

10.1 Regulation statement

This project will be conducted according to the principles of the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, October 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO) of Dutch law.

10.2 Recruitment and consent

Recruitment will take place among gonorrhea patients visiting the STI outpatient clinic of the Public Health Service (GGD) in Amsterdam and will be performed by the local team of nurses and physicians. If a patient is interested in participation, further information will be given either by the research nurse or the study coordinator both verbally and in a written patient information brochure. Two main pathways in establishing the diagnosis of a Ng infection can be distinguished depending on the reason for consultation. As is shown in figure 7, part of the gonorrhea patients consulting the STI clinic have symptoms suggestive for gonorrhea. In these patients a gram stain is done immediately. If the gram stain is positive for gram negative diplococci (suggesting *Neisseria gonorrhoeae)* a preliminary diagnosis is established, and these patients are immediately treated for gonorrhea. Gonorrhea patients without symptoms have to wait 2-10 days for the results of the NAAT before they receive treatment. For the first group we offer a time period of 2 hours to read the patient information brochure and consider participation of the study. The latter group will receive a digital version of the patient information brochure, together with the gonorrhea diagnosis and the advice to make an appointment for treatment at the STI clinic. The latter patient group will have at least 24 hours to consider participation.
Written informed consent will be obtained by the research nurse or the study coordinator. The patient information brochure, including the informed consent form, is attached to this document in the appendices (E1).

Besides the STI outpatient clinic of the Public Health Service (GGD) in Amsterdam, volunteers for the PK substudy will also be recruited at the Amsterdam University Medical Centers (AUMC) and the University for Applied Sciences Amsterdam (HvA) using posters with the recruitment text. If a volunteer is interested in participating in this substudy we will give the person further information verbally and by a written participant information brochure with the informed consent attached. We also make an appointment for the inclusion visit. At this visit informed consent forms will be signed.

10.3 Objection by minors or incapacitated subjects

Not applicable.

10.4 Benefits and risk assessments

Considering the pattern of development of resistance to previous first line treatment regimens for gonorrhea and the emergence of Ng strains with decreased susceptibility or even resistance to ESC, it is expected that gonorrhea may become untreatable in the near future. For this reason, there is an urgent need to find alternative treatment options. Previous research suggests that ertapenem,

fosfomycin and gentamicin might be effective and safe options. However, this is not yet proven by well-designed and robust trials in uncomplicated anogenital gonorrhea cases. The most important risk for participants in this study is thus inefficacy of one of the treatment options, and therefore a delay in the administration of efficacious treatment. To minimize this risk, we have installed a DSMB to perform an interim analysis on treatment efficacy. Due to disproportional numbers of treatment failure in the fosfomycin arm, we have preliminary terminated this arm. Furthermore, there is always a risk of adverse events in medication trials. Since ceftriaxone, ertapenem and gentamicin are registered and have been safely used for several indications for decades, we expect the risk of serious adverse events to be minimal. However, nephro- and ototoxicity are known side effects of gentamicin, in particular among patients receiving multiple (high) dosages of gentamicin and among patients with renal impairment. Although the effects of a single dose of gentamicin have not been structurally investigated, we do expect this risk to be low in our relatively healthy study population receiving a single intramuscular dose. The consequences of nephro- and ototoxicity are considered serious, therefore we will examine the renal function (by performing a point-of-care serum creatinin test) and symptoms of ototoxicity (by questionnaire) before and after the admission of treatment. We will exclude patients with renal impairment defined as an eGFR ≤50 ml/min (Cockroft-Gault). A disadvantage of participation is the administration of an additional intramuscular injection. Albeit the risk of pain/bleeding/infection at injection site is very low, it is increased as a result of two IM injections instead of one. In conclusion, the most important benefit for anyone at risk for STIs is the aim to assure treatment options for gonorrhea in the near future. A benefit for participants is a TOC (not otherwise done) and thus assurance of bacterial eradication.

10.5 Compensation for injury

The sponsor has a liability insurance which is in accordance with article 7 of the WMO. The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study. The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

10.6 Incentives

All 60 healthy volunteers that consent to participate in the PK substudy will receive a reimbursement of 100 euros to compensate for the 5 visits within 24 hours.

All 60 gonorrhea participants that consent to participate in the PK substudy will receive a reimbursement of 20 euros to compensate for the extra visit 30-90 minutes after treatment administration.

11. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

11.1 Handling and storage of data and documents

Clinical data and patient samples will be stored according to the standard procedures of the STI outpatient clinic. Electronic patient files are accessible to all medical personnel of the STI outpatient clinic with access and codes to the computer system. All data collected for the trial in particular [e.g. general medical history, in- and exclusion criteria (T0), adverse events and new medication use (T7)] will be directly entered in a digital database using a unique de-identified project ID, which is allocated to each participant at study inclusion. These data are thus not accessible for all medical personnel of the STI outpatient clinic, but only for the NABOGO research team. The data entry system has a detailed audit trail, tracking who modified the data and when they were modified. All research data will be stored for 15 years. The study coordinator will maintain a list linking study ID's to electronic patient file identifiers. This list will be kept in a locked filing cabinet with restricted access. Information gained from the data collection and analysis of this trial will be available for inspection on request by the participating physicians, the METC, the DSMB, the monitoring board, the funder and the regulatory health authorities.

11.2 Monitoring and Quality Assurance

Monitoring of accuracy and quality of the research data and performance of the research is compulsory for this study. The CRU of the AMC will organize and perform the monitoring. A detailed monitoring plan will be composed in collaboration with the CRU.

11.3 Amendments

A 'substantial amendment' is defined as an amendment to the terms of the METC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

All substantial amendments will be notified to the METC and to the competent authority. Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

11.4 Annual progress report

The investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

11.5 Temporary halt and (prematurely) end of study report

The sponsor will notify the accredited METC and the competent authority of the end of the study within a period of 90 days. The end of the study is defined as the last contact with the last patient (30 days after the last participant has received treatment). The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action. In case the study is ended prematurely, the sponsor will notify the accredited METC and the competent authority within 15 days, including the reasons for the premature termination. Within one year after the end of the study, the investigator will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC and the Competent Authority.

11.6 Public disclosure and publication policy

This trial will be registered at clinicaltrials.gov and EudraCT. It is our intention to publish study outcomes in the most appropriate peer-reviewed scientific journals and at scientific congresses. Both the sponsor and the subsiding party support the intention to disclose the results of this trial. Authorship will follow the guidelines defined by the international Committee of Medical Journal Editors.

12. STRUCTURED RISK ANALYSIS

12.1 Potential issues of concern

Ertapenem

a. Level of knowledge about mechanism of action

The mechanism of action of ertapenem is well known. Ertapenem is one of the β-lactam antimicrobials, it is rapidly bactericidal and derives its activity from binding to specific penicillin binding proteins (PBP) and subsequent blocking of cell wall synthesis[38].

b. Previous exposure of human beings with the test product

Ertapenem is widely used in patients with the following moderate to severe infections caused by susceptible bacteria: complicated intra-abdominal infections, complicated skin infections, community acquired pneumonia, complicated urinary tract infections, acute pelvic infections[38]. In Europe, ertapenem is only registered as intravenous treatment, however in the USA it is safely administered either intravenously and intramuscularly for the previously mentioned indications [37]. As far as we know, there are no clinical trials investigating the efficacy and safety of ertapenem in patients infected with *Neisseria gonorrhoeae*.

c. Can the primary or secondary mechanism be induced in animals and/or in *ex-vivo* human cell material?

Not applicable.

d. Selectivity of the mechanism to target tissue in animals and/or human beings

Non-clinical data reveal no special hazard for humans based on conventional studies of safety, pharmacology, repeated-dose toxicity, genotoxicity and toxicity to reproduction and development. Decreased neutrophil counts, however, occurred in rats that received high doses of ertapenem, which was not considered a significant safety issue. Long-term studies in animals have not been performed in order to evaluate the carcinogenic potential of ertapenem [38].

e. Analysis of potential effect

Previous research showed that ertapenem 1000mg is a safe, well tolerated and effective agent against urinary tract infections [62-66]. Based on PK/PD simulations using MIC values of Ng strains from the STI clinic in Amsterdam, we expect that ertapenem 1000mg single dose will be effective against gonorrhea as well (unpublished data, June 2014, correspondence with R.A.A. Mathot). The following serious adverse events are reported rarely: antibiotic-associated pseudomembranous colitis and seizures (in elderly patients and those with pre-existing central nervous system disorders). Other, commonly reported (>1/100 - <1/10), adverse events are: diarrhoea, nausea, rash, headache, elevations in ALT, AST, alkaline phosphatase and platelet count. Of note, these data are based on patients using multiple doses of ertapenem, it is thus expected that the prevalence and severity of adverse events will be lower in our patients. The only explicit contra-indication is an anaphylactic reaction to any β-lactam antibacterial agent [38].

f. Pharmacokinetic considerations

Ertapenem is highly bound to plasma proteins. In healthy young adults the plasma half-life is 4 hours. There are inadequate data on the safety and efficacy of ertapenem in patients with advanced renal impairment to support a dose recommendation. Therefore, ertapenem should not be used in these patients [38].

g. Study population

The average population consulting the STI outpatient clinic in the city center of Amsterdam is young and has limited health concerns. A large proportion of this population is HIV positive, but in most of them this is well treated.

h. Interaction with other products

Co-administration of valproic acid with carbapenem agents may result in a decreased level of valproic acid falling below therapeutic range. This can lead to inadequate seizure control.

i. Predictability of effect

There are no markers available to predict the effect of ertapenem.

j. Can effects be managed?

In case of treatment failure, participants receive a doubled dose of ceftriaxone (1000mg) plus azithromycin 1000mg. Research subjects can always contact the STI outpatient clinic and the study coordinator in case of any questions or uncertainties. At the STI outpatient clinic a case with emergency medication (for instance, for anaphylactic reaction or seizures) is available. If necessary participants can be referred to a specialist in the hospital.

Fosfomycin

a. Level of knowledge about mechanism of action

The mechanism of action of fosfomycin is well known. Fosfomycin is a fosfonic acid derivate which exhibits bactericidal activity by inhibiting cell wall synthesis through inhibition of fosfoenolpyruvate transferase. This enzyme is involved in the first phase of peptidoglycan synthesis in both Gram-negative and Gram-positive bacteria. At the same time, fosfomycin inhibits adhesion of bacteria to the bladder mucosa and consequently prevents re-infection. Because of the distinctive mechanisms of fosfomycin, there is minimal cross-resistance with other antimicrobials [60-62].

b. Previous exposure of human beings with the test product

Fosfomycin-Trometamol is widely used in humans for the treatment of urinary tract infections and is a registered medicinal product at EMA. Three clinical studies have been performed to study the effect of fosfomycine in uncomplicated gonococcal urethritis. In these studies no severe adverse events were reported [14, 30, 63].

c. Can the primary or secondary mechanism be induced in animals and/or in *ex-vivo* human cell material?

Not applicable.

d. Selectivity of the mechanism to target tissue in animals and/or human beings

Oral fosfomycin is metabolized after absorption to the free acid fosfomycin, which will be eliminated in urine and faeces. It is mainly distributed to the kidneys, bladder wall, prostate and seminal vesicles. Preclinical data, based on conventional studies with single and repeated toxicity studies, genotoxicity studies and reproduction and development studies do not reveal any special risks for human beings.

e. Analysis of potential effect

Common adverse events of fosfomycin are mild and often self-limiting, including diarrhea, nausea, abdominal pain and headache. The only explicit contra-indication is an anaphylactic reaction to fosfomycin in the past [61, 64].

f. Pharmacokinetic considerations

The maximal plasma concentration is reached in 2 hours after intake. The half-life is 6 hours. fosfomycin is completely eliminated by the kidneys. Higher levels of fosfomycin concentration are found in urine than the MIC in peripheral blood samples after 24-48 hours[64].

g. Study population

The average population is young and has limited health concerns.

h. Interaction with other products

The serum concentration and urinary excretion of fosfomycin are decreased by co-administration of metoclopramide, but otherwise it has limited drug interactions[61]. A simultaneous intake with food delays fosfomycin absorption, following a decrease in peak plasma and urine [64].

i. Predictability of effect

There are no markers available to predict the effect of fosfomycin.

j. Can effects be managed?

Research subjects can always contact the STI outpatient clinic and the study coordinator in case of any questions or uncertainties. At the STI outpatient clinic a case with emergency medication (for instance, for anaphylactic reaction or seizures) is available. If necessary participants can be referred to a specialist in the hospital.*Gentamicin*

a. Level of knowledge about mechanism of action

The mechanism of action of gentamicin is well known. Gentamicin is a bactericide antibiotic that inhibits the synthesis of proteins by binding to the 30S subunit of the bacterial ribosome. Gentamicin is known to be effective in a large amount of pathogen Gram-positive and Gram-negative bacteria [43].

b. Previous exposure of human beings with the test product

Gentamicin is a widely used antibiotic for several indications and is a registered medicinal product at EMA. In different developing countries, gentamicin is being used for the treatment of gonorrhea for decades [14, 15, 67].

c. Can the primary or secondary mechanism be induced in animals and/or in *ex-vivo* human cell material?

Not applicable

d. Selectivity of the mechanism to target tissue in animals and/or human beings

After intramuscular admission, gentamicin is well distributed to body tissue and body fluids. However, low concentrations are found in cerebrospinal liquor, sputum and pleural and peritoneal fluids. Gentamicin passes both peritoneal and placental membranes. Preclinical safety research did not reveal any specific risks for human beings [43].

e. Analysis of potential effect

See 12.1b. Additionally, Felarca et al. found the adequate (efficient and safe) dose of gentamicin for the treatment of gonorrhea[39], subsequently several studies have been enrolled using a dose of 240-280mg gentamicin IM with cure rates between 94-100%[18, 40-42]. Also, the susceptibility for gentamicin of *Neisseria gonorrhoeae* strains is widely investigated in Ng strains from different regions[14, 18, 42, 68-71]. Limited research on adverse events of gentamicin in patients with gonorrhea showed no nephrotoxicity and ototoxicity after a single dose intramuscular injection [39-42, 72].

f. Pharmacokinetic considerations

Gentamicin is not metabolized in the human body and it is eliminated in its active form. In patients with a normal renal function, the half-life is between 2-3 hours. In patients with impaired renal function, the half-life is reduced based on the remaining renal function. Toxic dosages can cause renal impairment and/or neurological damage [43].

g. Study population

The average population consulting the STI outpatient clinic in the city center of Amsterdam is young and has limited health concerns. A high number of this population is HIV positive, but in most of them this is well treated. Therefore, we expect that the rate of participants with renal impairment or other chronic diseases will be low. However, because of the limited knowledge on adverse-events after one single dose of gentamicin, the renal function will be tested on forehand in all possible participants and people with renal impairment will be excluded from the study. Also pregnant women will be excluded from this study, since little is known on the effects of pregnancy [43].

h. Interaction with other products

There are no drug-interactions known with gentamicin. However, attention needs to be paid to nephro- and ototoxic medicines, because this effect could be increased when simultaneously using gentamicin [43]. For this reason, we will evaluate the renal function and we will exclude participants with renal impairment.

i. Predictability of effect

There are no markers in order to predict the effect of gentamicin.

j. Can effects be managed?

In case of treatment failure, participants receive a doubled dose of ceftriaxone (1000mg) plus azithromycin 1000mg . Research subjects can always contact the STI outpatient clinic and the study coordinator in case of any questions or uncertainties. At the STI outpatient clinic a case with emergency medication (for instance, for anaphylactic reaction or seizures) is available. If necessary participants can be referred to a specialist in the hospital.

12.2 Synthesis

Ceftriaxone

Ceftriaxone is the standard therapy for gonorrhea infections in the Netherlands, given the current efficacy-rate of 100% and the mild adverse events that are reported. For this reason, chapter 12.1 is not indicated for this product. Furthermore, in this trial Ceftriaxone will not cause any risks in comparison to the general population with gonorrhea. Consequently there is no need for extra measures to reduce expected risks.

Ertapenem

Ertapenem is safely used for several indications worldwide both in intramuscular and intravenous form. In Europe ertapenem is not yet registered as intramuscular injection, however it is safely used intravenously. We do not expect any harm from a single intramuscular dose in our young and relatively healthy population. Because of the influence of Ertapenem on the valproic acid plasma levels, patients using valproic acid are excluded from this trial.

Fosfomycin

Fosfomycin is safely used for several indications worldwide. A few clinical studies with (either intramuscular or oral) Fosfomycin have been performed in patients with uncomplicated gonococcal urethritis, none of these studies reported any severe events. Participants of this trial are assured not to take metoclopramide at the first 7 days. In case of the intake of metoclopramide on the day of inclusion or in case of the intention to take metoclopramide in the following days, patients are excluded from this study.

Gentamicin

Since a single intramuscular dose of gentamicin is used for gonorrhea in Malawi [14, 73] for decades and effectiveness is reported in different studies, we can expect that it is an efficient and safe product for this indication. Because of limited knowledge on the prevalence of nephro- and ototoxicity as a result of the single dose gentamicin, renal function will be measured before treatment is given and people with an impaired renal function will be excluded from this study. This is determined by a point-of-care serum creatinin test before administration of treatment. Additionally, at the follow-up visit 7-14 days after treatment, renal function will again be tested in order to evaluate the effects of a single dose gentamicin on renal function. Also deafness and balance disorder will be evaluated before and after treatment in order to look for ototoxicity after a single dose injection of gentamicin.

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