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

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

a double-blind randomized clinical non-inferiority trial

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

PROTOCOL TITLE: New antibiotic treatment options for uncomplicated anogenital gonorrhoea infections – a double-blind randomized clinical 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**
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***

PP	**Per Protocol analysis**
POC	**Point-of-Care (test)**
RCT	**Randomized Controlled Trial**
(S)AE	**(Serious) Adverse Event**
SPC	**Summary of Product Characteristics (in Dutch: officiële productinfomatie IB1-tekst)**
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 subsidising party.**
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) among *Neisseria gonorrhoeae* (Ng) is a major public health concern. With no alternative antimicrobial treatment options for gonorrhoea and only a few new drugs in the development pipeline, it is important to test existing antibiotics for their efficacy in gonorrhoea treatment.

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

Study design: A double blinded randomized controlled non-inferiority trial with four treatment arms.

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

Intervention: Fivehunderd forty-eight patients are randomly assigned to one of four treatment arms. They will receive either ceftriaxone 500mg intramuscularly (IM) or ertapenem 1000mg IM or gentamicin 5mg/kg IM with a maximum of 400mg (in two doses) supplemented with an oral placebo, or receive fosfomycin 6g oral suspension supplemented with an intramuscular placebo.

Main study endpoint: The bacterial eradication capacity of the 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 gonorrhoea in adults aged 15-49 years, gonorrhoea is the second most bacterial STI worldwide. Persistent Ng infections may cause severe reproductive tract inflammation and irreversible damage with infertility as a result. Moreover gonorrhoea also increases the risk of human immunodeficiency virus (HIV) transmission. When untreatable gonorrhoea becomes reality, an increase will be seen in both the burden of disease and costs for society.

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

The burden of participation to this study comprises a double instead of a single intramuscular injection, because the injectable volume of gentamicin will be too large (6-10 ml) for a single injection. At the same time all participants receive an oral suspension, a finger prick to draw blood for point-of-care (POC) serum creatinin test, one extra visit at day 7 for follow-up with another NAAT and keeping a diary between treatment and follow-up visit after 7 days. A subset of approximately 160 participants

will additionally be asked to give two to three blood samples (total volume: 9 – 13.5 ml) for the purpose of estimating the population pharmacokinetics, which will be used for a PK/PD analysis in order to aid the optimization of treatment regimes.

1. INTRODUCTION AND RATIONALE

With 78 million new cases of gonorrhoea in adults aged 15–49 years, gonorrhoea is the second most common bacterial sexually transmitted infection worldwide (STI)[1]. Persistent infections may cause severe genital and reproductive tract inflammation and irreversible damage with infertility as a result. Moreover, gonorrhoea 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 gonorrhoea due to resistance to extended spectrum cephalosporins (ESCs) in 10 countries. ESCs are the treatment of last resort for gonorrhoea. 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, gonorrhoea may become untreatable unless new drugs become available or existing drugs that are presently not used for treatment of gonorrhoea are tested for their efficacy in gonorrhoea infections[5-7].

The threat of untreatable gonorrhoea infections 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 gonorrhoea 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 gonorrhoea is approximately 440,000[8]. Antimicrobial resistance in gonorrhoea 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 gonorrhoea using the registered drugs, ertapenem, fosfomycin and gentamicin, which have not yet been proven clinically effective against gonorrhoea. 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 gonorrhoea 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 gonorrhoea 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 gonorrhoea 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 gonorrhoea.

We propose a non-inferiority, double-blinded randomized controlled trial to determine if one or more of the experimental treatment options against uncomplicated anogenital gonorrhoea are a good alternative for the current gold standard therapy in the Netherlands (500 mg ceftriaxone intramuscular (IM)), based on a test of cure, disappearance of symptoms and frequency and severity of adverse events.

It is important to consider that the efficacy of an antimicrobial agent is dependent on the relationship between the MIC for the microorganism and the exposure of the microorganism 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].

2. OBJECTIVES

Primary objectives

To determine the bacterial eradication capacity of ertapenem, fosfomycin and gentamicin compared to the reference treatment (ceftriaxone) in uncomplicated anogenital gonococcal infections (at one included infection site) by molecular test of cure (TOC) 7-14 days after treatment.

Secondary objectives

1. To determine the bacterial eradication capacity of the experimental treatment options (ertapenem, fosfomycin 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 gonorrhoea) 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 *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.
5. 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.
6. To determine the clinical and demographic predictors for treatment failure (see chapter 7 for definition).
7. To determine the population pharmacokinetics of ceftriaxone, gentamicin and ertapenem administered intramuscularly, and fosfomycin administered orally.

3. STUDY DESIGN

A double-blinded randomized controlled single center non-inferiority trial with three experimental arms and one reference arm. The expected duration of the study is 36 months, of which 18 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.
All participants are requested to participate in a subset for a pharmacological analysis as well until we have included 160 participants. Following randomization we aim to include approximately 40 participants in each treatment arm for this additional subset.

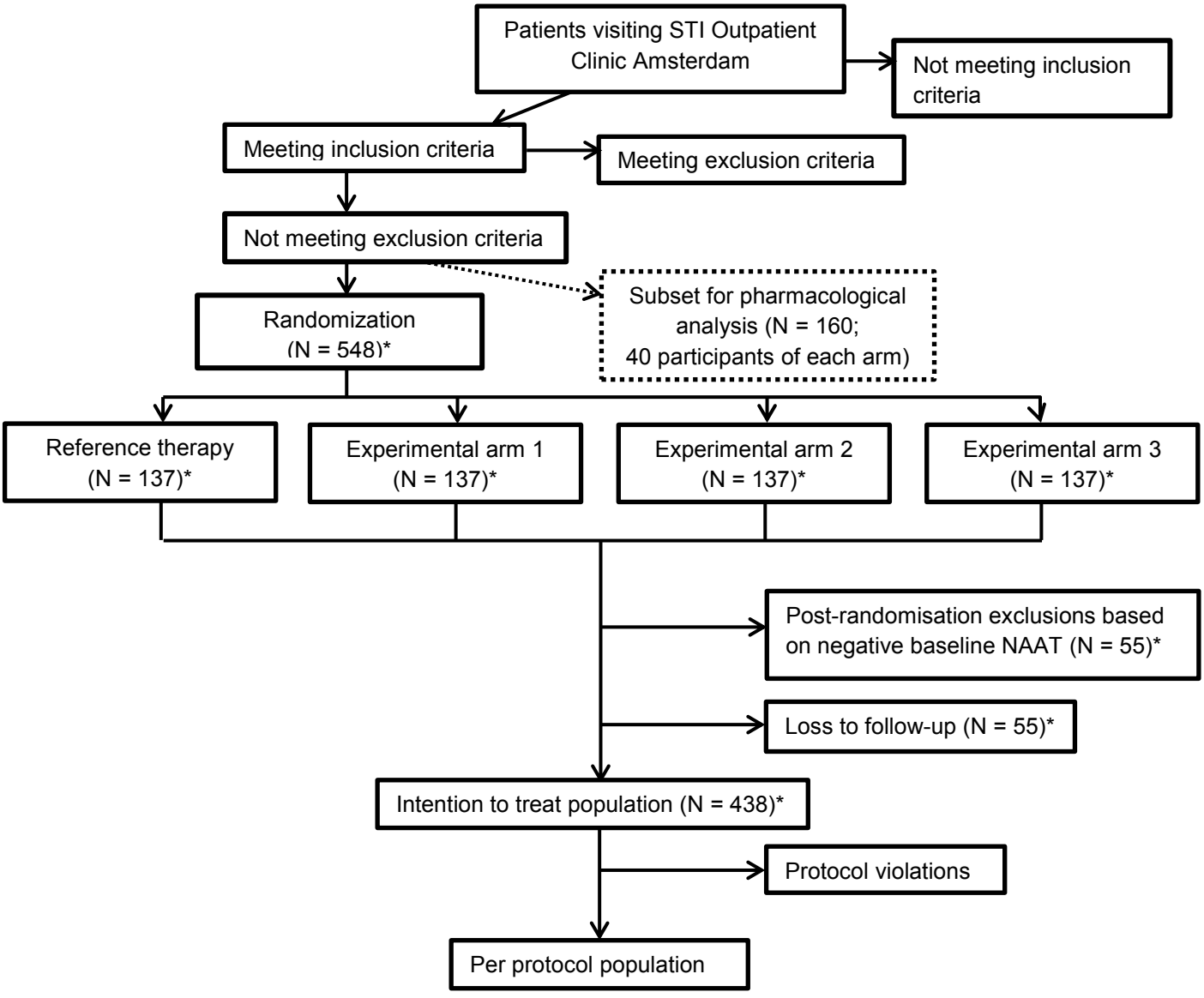

Figure 1. Flow chart of study design and sample size.

4. STUDY POPULATION

4.1 Study population

Patients of the STI Outpatient Clinic Amsterdam with an uncomplicated anogenital Ng infection will be included in this study. At the STI clinic in Amsterdam we diagnosed 2230 new gonorrhoea 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 gonorrhoea 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].

4.2 Inclusion criteria

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
- Able to swallow liquid and accept intramuscular injections

4.3 Exclusion criteria

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 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, ertapenem, fosfomycin or 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
    - systemic metoclopramide
- 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 intention to treat analysis (ITT):

- Negative result of Ng NAAT of sample collected on T0 (the day of treatment):
    1. Negative NAAT in spite of positive gram stain.
    2. Positive NAAT on prestudy 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 ITT.

Exclusion of per protocol analysis (PP):

- Exclusion of ITT
- Use of non-study related antibiotics after inclusion prior to TOC visit
- Other protocol violations

4.4 Sample size calculation

To calculate the sample size for this non-inferiority trial we used the R function nBinomial of the package gsDesign. This function computes sample size using the method of Farrington and Manning for a trial to test the difference between two binomial event rates[25]. 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), 90% power, two-sided α-level of 0.0083 (including Bonferroni adjustment for three comparisons). This results in a sample size of 109 participants per treatment arm. Considering a loss-to-follow-up rate of 10% (participants not returning for TOC or exclusion criteria) and considering a post hoc exclusion of 10% due to false-positive gram stains and self-clearance, we require 137 included participants per treatment arm. Thus, a total of 548 participants should be included.

In order to estimate the population pharmacokinetics, we will measure blood concentration of the administered antimicrobial in 160 participants at two or more various time points[26]. As a result of the randomization, it is expected that approximately 40 participants will be included per treatment arm. With the collected data reliable population pharmacokinetic-pharmacodynamic models will be derived for Monte Carlo simulations.

4.4.1. Recruitment duration

At the STI clinic in Amsterdam we diagnosed 1667 new anogenital gonorrhoea 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 548/25 = 22 months.

4.5 Patient registration

Inclusion of participants will be performed by either a research nurse or 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 either one of three experimental therapies (intramuscular gentamicin and ertapenem and oral fosfomycin) or the reference therapy (intramuscular ceftriaxone). Participants and the treating health professional will be blinded for the treatment allocation. For blinding purposes, those receiving intramuscular therapy (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) will receive a placebo oral solution and; those receiving oral therapy (fosfomycin) will receive two placebo intramuscular injections.

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 an objectified treatment failure (see chapter 7.1.1 for definition), patients are retreated 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, in the case of clinical treatment failure it is possible that one received ceftriaxone 500mg. Besides, it is possible that antimicrobial susceptibility testing at inclusion indicates decreased susceptibility or resistance to ceftriaxone. For this reason, we chose to double the dose of ceftriaxone and to add azithromycin to the escape medication regime.

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.
- 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).
- Placebo oral suspension: orange lemonade.

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 gonorrhoea. 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) [27]. In Europe, ertapenem is not yet registered as intramuscular injection, however it is safely used intravenously [28]. 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 [13, 29, 30]. 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, 31-34] in patients with gonorrhoea 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 [35]. 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 [27]. Fosfomycin oral suspension is available and preferred in the light of patient comfort and time management.

For blinding purposes there are two treatment modalities: either therapeutic treatment will be administered intramuscularly (ceftriaxone, ertapenem or gentamicin) and placebo (orange lemonade) will be administered orally, or therapeutic treatment will be administered as an oral suspension (fosfomycin) and placebo (0.9% NaCl) will be administered intramuscularly. Solutions of ceftriaxone, ertapenem and gentamicin are colorless to light-yellow and have a similar visual appearance as placebo. Fosfomycin oral solution contains an orange/mandarin flavoring agent. Taste is comparable to the placebo orange lemonade.

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 [36-48], and MIC data of Ng strains isolated in Amsterdam Dosage of ceftriaxone is according to current Dutch STI guidelines [49].

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.

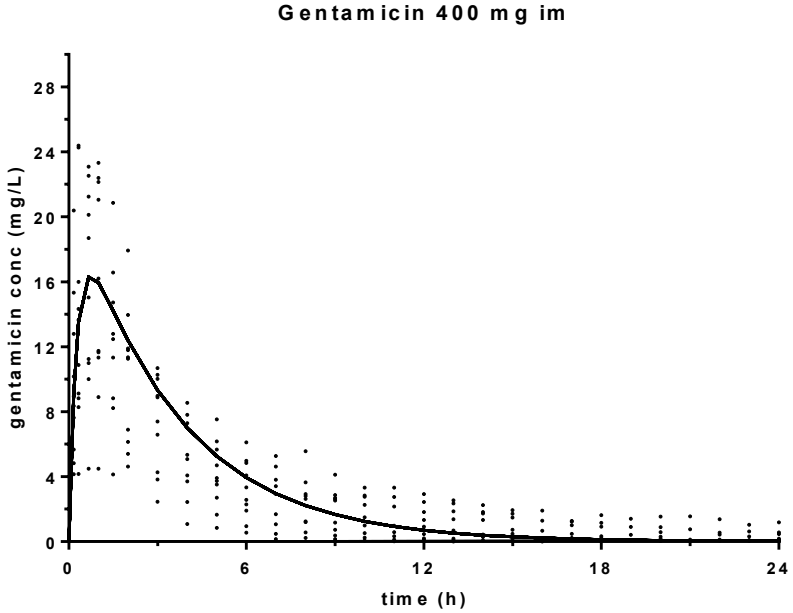

Figure 2: Monte Carlo simulation of 5 mg/kg (max 400 mg) gentamicin IM

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)

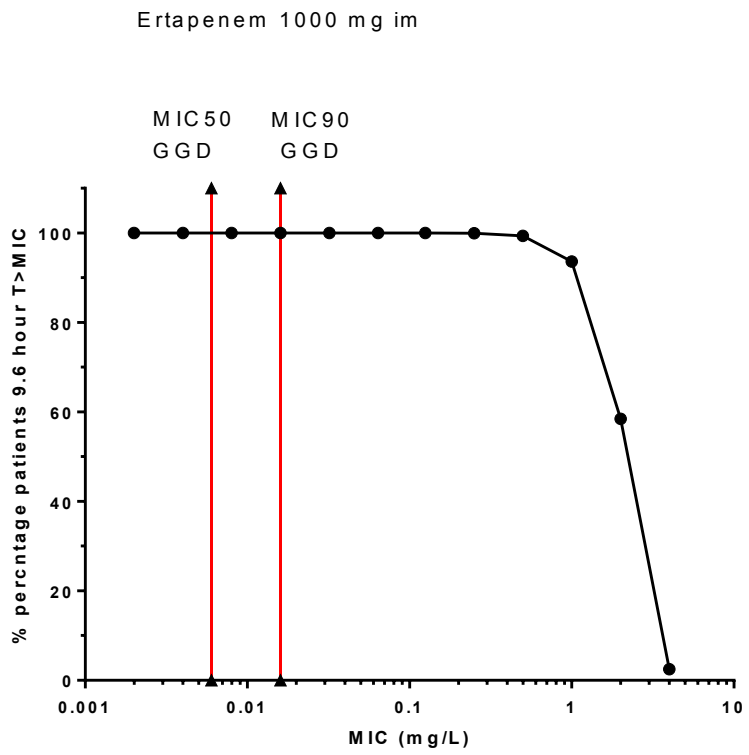

Figure 3: Monte Carlo simulation of 1000 mg ertapenem IM

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 [29].

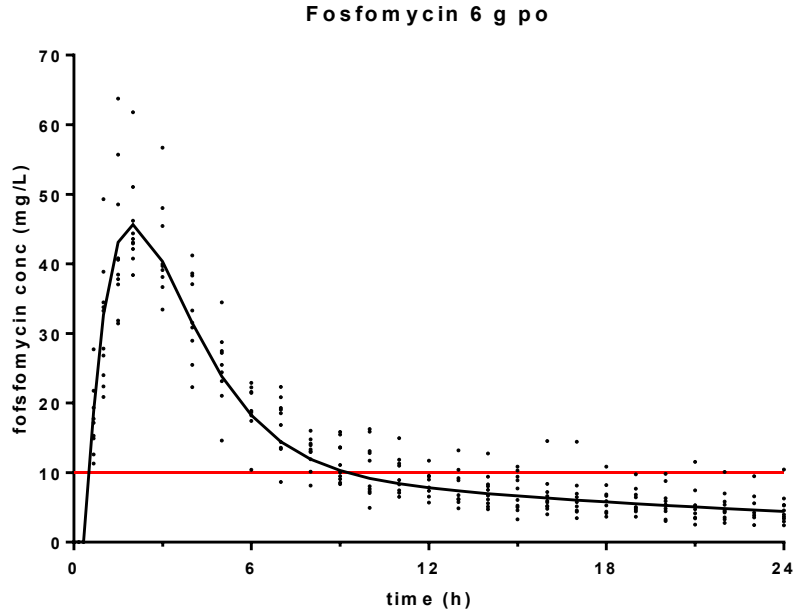

Figure 4: Monte Carlo simulation of 6 gram oral fosfomycin

6.7 Preparation and labeling of Investigational Medicinal Product

This study will be performed in a double-blinded 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 from the STI clinic. When a patient is included in the study, this independent physician 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 doctor with the participant study ID linked to the allocated study arm. Four to five medical doctors from the STI clinic will be trained to function as an independent treatment preparer.

6.8 Drug accountability

Ceftriaxone, fosfomycin 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 and for oral placebo we will use orange lemonade (private label of Albert Heijn).

7. METHODS

7.1 Study endpoints

7.1.1 Primary endpoint

Bacterial eradication of Ng infection at the included 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).

An exceptional group contains participants who receive escape medication because of a suspected persistent Ng infection based on persisting symptoms and a positive Gram stain 3-6 days after treatment administration. These cases will be considered as 'treatment failures' without the performance of a test of cure, because a NAAT will not be reliable less than 7 days after treatment administration.

A positive test of cure can be the result of a persistent infection and thus failure of the administered treatment, or can be a result of a re-infection. Re-infection could be either with the same Ng species (usually from the same partner(s)) or with another Ng species. In order to be able to differentiate between a persistent infection (treatment failure) or a re-infection with another Ng species (treatment success) we will perform genotyping of the Ng strains [50]. If we find two similar Ng strains we cannot differentiate between a persistent infection or a re-infection with the same strain, for this reason we follow the result of the test of cure in defining treatment failure or success in the primary analysis.

7.1.2. Secondary endpoints

1. Bacterial eradication of Ng infection at the included 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) 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* MICs 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.
    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 1:1:1:1 ratio to the four 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 (8-12 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 perform allocation.

7.2.2 Blinding

This study will be performed in a double-blinded fashion. The patient, study nurse and the study coordinator 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 and a liquid substance that they will drink under direct observation at the day of inclusion.

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 is also an indication for deblinding the treatment allocation in a particular participant.

7.3 Study procedures

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

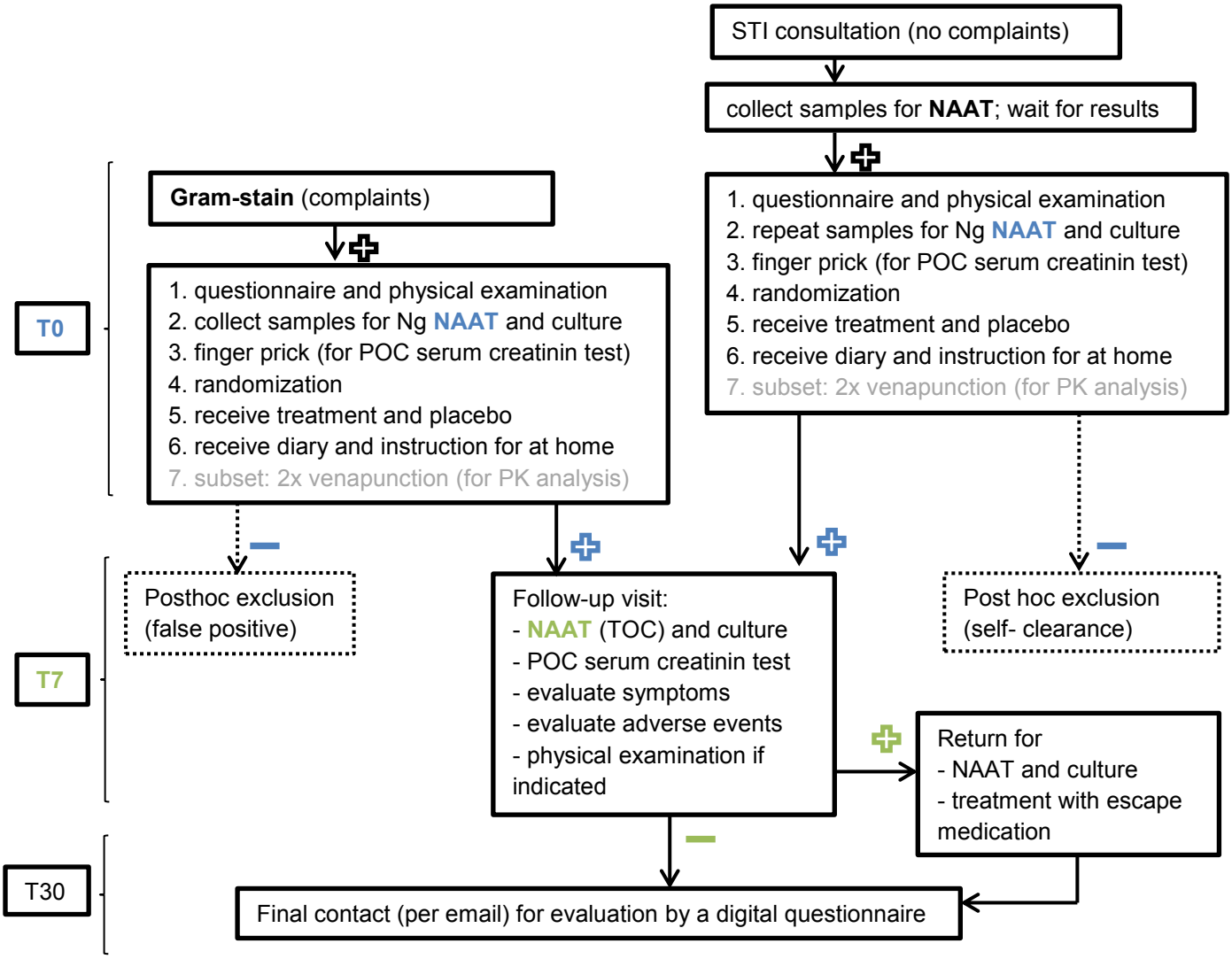

Figure 5. Flowchart of study procedures

Participants who contact the STI clinic in less than 7 days after treatment administration because of persisting or worsening symptoms, will be evaluated by the study coordinator. If indicated, a gram stain will be repeated. If the gram stain is negative after treatment administration, 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 eventually the study physician decides whether to treat with escape medication or not. Ng-RNA may still be detectable within 7 days after treatment, even when the infection has been effectively treated [51]. This means that a positive NAAT within 7 days is not a reliable indication of treatment failure. If escape treatment is given and the NAAT is negative, this case will be considered as treatment failure in the intention to treat (ITT), but will be excluded from the per protocol analysis. When escape treatment is given and the culture is positive, this case will be regarded as a treatment failure in both the ITT- and per protocol (PP) analysis.

Each participant (until a total of 160 participants is reached) will be requested to return for the collection of a peripheral blood sample at two or three random time points distributed over the following options: 0.5, 1, 2, 4, 6, 8 or 24h after treatment administration. With the collected data a reliable population pharmacokinetic model will be derived for Monte Carlo simulations. For the pharmacokinetic study a maximum of 13.5 ml blood will be obtained by venipuncture.

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).

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

- Anal, pharyngeal and vaginal swabs and urine for RNA-based NAAT (Aptima Combo 2 assay) at T0, at T7, and in case of treatment failure another NAAT just before the administration of escape medication and 7-14 days later a second test of cure. 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[52]. It has recently been recommended to perform the test no sooner than 7 days after treatment [51].
- Anal, cervical, pharyngeal and urethral swabs for culture at T0, at T7 and in case of treatment failure additional cultures will be performed just before the administration of escape medication and 7-14 days afterwards.
- 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.
- 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.
- Finger prick to collect a drop of blood (around 30 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).

- In a subset of participants, a venapunction to collect 4,5 ml blood at two or three time points (0.5, 1, 2, 4, 6, 8 or 24h after treatment administration) in which plasma concentration of the antibiotics will be measured. The maximal volume is 13.5 ml.

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.

7.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons (e.g. an urgent medical condition which requires treatment with a known interaction with one of the study drugs).

7.5 Replacement of individual subjects after withdrawal

This longitudinal study has a relative short follow-up period. Patients who are lost to follow-up or who are post-hoc excluded from analyses (see section 3, 4.3 and 4.4) will not be replaced if the proportion of included participants lost and/or post-hoc excluded does not exceed the expected 20% (see section 3, 4.3 and 4.4). If it does exceed the expected 20% when recruitment is still ongoing, we will replace them by new inclusions until at least 438 analysable patients are included.

7.6 Follow-up of subjects withdrawn for treatment

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

7.7 Premature termination of study

If during interim analysis a treatment success is found among < 60% of the participants in one treatment arm, this arm will be terminated. Besides, we will stop a treatment arm 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 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 gonorrhoea.
- 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

Descriptive demographic and clinical data (including symptoms and signs) will be compared between the treatment groups at baseline.

9.1 Primary study parameters

If a single patient is infected with *Neisseria gonorrhoeae* at more than one anatomical site, only one site will be included in the 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 of multiple infected sites will be: 1) cervical infections, 2) urethral infections and 3) anal infections.

Both a per protocol analysis (PP) and an intention to treat analysis (ITT) will be performed. In this non-inferiority trial, the primary approach to between group comparative analysis would be by per protocol analysis. An intention to treat analysis will be less conservative because of the risk of dilution in treatment effect and consequently an increased risk of false rejection of the null hypothesis. However, an ITT analysis is important in order to preserve the value of randomization and better reflect clinical practice.

For the evaluation of the primary clinical outcome variable (treatment success), the risk difference between each of the experimental treatments and the reference treatment will be calculated. The confidence interval around this risk difference will be calculated using the R function ci.pd of the R package Epi. This package uses the Newcombe method to estimate the interval for the difference between independent proportions [53]. If the lower limit of the confidence interval around the risk difference between an experimental treatment and the reference treatment does not exceed -10% (the predefined non-inferiority margin), the experimental treatment will be declared to be non-inferior to the reference treatment. Following Bonferroni correction for three comparisons as a result of the four treatment groups, we will calculate a two-sided 98.3% confidence interval around the risk difference.

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.

Adverse event analysis will be descriptive. Frequency counts and percentages of the prespecified main categories of AEs will be presented by treatment arm.

Explorative analyses will be performed to assess determinants of treatment failure and the existence of adverse events using univariate logistic regression analysis. Variables that are associated with treatment failures or adverse events will be included into a multivariable logistic regression model.

Antimicrobial susceptibility (in MIC) will be measured for all Ng strains obtained the day of inclusion (T0) and at the TOC visit (T7). Drug concentrations will be assessed in peripheral blood samples collected from 160 participants at two or three different time points distributed over the following time points: 0.5, 1, 2, 4, 6, 8 or 24 hours after treatment gift. The 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

One interim analysis will be performed by the Data Safety Monitoring Board (DSMB) in order to timely tackle the exposure to a significantly less effective agent. After one third of all planned inclusions, the proportion of treatment failures in the three experimental treatment arms will be calculated. When in one arm 40% or more of the participants were not successfully treated (i.e. less than 60% have a negative TOC), this agent will be considered ineffective and the arm will be terminated. This proportion of treatment failure is considered clinically unacceptable by the research team. When assuming a cure rate of 98% in the reference treatment, we calculated that with a number of 36 participants per treatment arm (approximately one third of the final number of participants per treatment arm), the 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 -52% to -24%. This would mean that the experimental treatment is highly significantly worse than the reference treatment. The upper limit is even far under the non-inferiority margin of -10%.

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 gonorrhoea 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 5, part of the gonorrhoea patients consulting the STI clinic have symptoms suggestive for gonorrhoea. 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 gonorrhoea. Gonorrhoea 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 gonorrhoea 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, are attached to this document in the appendices (E1).

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 regimes for gonorrhoea on one side and, the emergence of Ng strains with decreased susceptibility or even resistance to ESCs on the other side, it is expected that gonorrhoea 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 gonorrhoea 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 will install a DSMB to perform an interim analysis on treatment efficacy. In the case of disproportional numbers of treatment failure in a treatment arm, we will preliminary terminate this arm.

Furthermore, there is always a risk of adverse events in medication trials. Since ceftriaxone, ertapenem, fosfomycin 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 by an eGFR ≤50 ml/min (Cockroft-Gault). A disadvantage of participation is the administration of an additional intramuscular injection and an oral suspension. 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 gonorrhoea 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 160 participants that consent to participate in the subset for the pharmacological analysis will receive a reimbursement of 30 euros to compensate for the inconvenience of 2 – 3 vena punctures and 1 – 3 return visits within the first 24 hours.

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 gained from the study questionnaires will be stored in a separate file using a unique project ID, which is allocated to each participant at study inclusion. 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[28].

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[28]. 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 [27]. 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 [28].

e. Analysis of potential effect

Previous research showed that ertapenem 1000mg is a safe, well tolerated and effective agent against urinary tract infections [54-58]. 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 gonorrhoea 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 [28].

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 [28].

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 [59-61].

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 [13, 29, 62].

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

The previously mentioned clinical trials investigating the efficacy of multiple dosed fosfomycin in patients infected with *Neisseria gonorrhoeae*, showed promising cure rates [13, 29, 62]. Based on Monte Carlo simulations with antimicrobial susceptibility data of Ng, it is calculated that single-dosed fosfomycin in a dose of 6g is potentially effective (unpublished data, June 2014, correspondence with R. Mathot or C. Wind). 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 [60, 63].

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[63].

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.

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[60]. A simultaneous intake with food delays fosfomycin absorption, following a decrease in peak plasma and urine [63].

i. Predictability of effect

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

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.

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 [35].

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 gonorrhoea for decades [14, 15, 64].

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 [35].

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 gonorrhoea[31], subsequently several studies have been enrolled using a dose of 240-280mg gentamicin IM with cure rates between 94-100%[18, 32-34]. Also, the susceptibility for gentamicin of *Neisseria Gonorrhoeae* strains is widely investigated in Ng strains from different regions[14, 18, 34, 65-68]. Limited research on adverse events of gentamicin in patients with gonorrhoea showed no nephrotoxicity and ototoxicity after a single dose intramuscular injection [31-34, 69].

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 [35].

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 [35].

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 [35]. 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 gonorrhoea 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 gonorrhoea. 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 gonorrhoea in Malawi [14, 70] 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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