Statistical Analysis Plan

Efficacy of screening STIs in MSM

GonoScreen

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Author: Jozefien Buyze
Biostatistician, Institute of Tropical Medicine, Antwerp, Belgium

Approved by: Coordinating Investigator
Chris Kenyon

Date: ____ / _____ / _____

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Table of Contents

1. Introduction

This Statistical Analysis Plan (SAP) provides a detailed and comprehensive description of the main, pre-planned analyses for the study "Does screening for gonorrhea and chlamydia affect the incidence of these infections in men who have sex with men taking HIV pre exposure prophylaxis (PrEP): a randomized, multicentre controlled trial". The purpose of this study is to establish if screening results in a clinically meaningful and cost-effective reduction in Ng/Ct incidence in MSM PrEP cohorts that could outweigh the increased risk of AMR development it confers. The study conduct is described in the Protocol.

These planned analyses will be performed by the statistician(s) at the Clinical Trials Unit of the Institute of Tropical Medicine (Antwerp) in collaboration with the research consortium. The analysis results will be described in a statistical analysis report, to be used as the basis of the primary research publications. This document describes statistical methods for the primary and secondary objectives of the study as defined by protocol. Additional analyses may be performed but are not covered by the current analysis plan.

This analysis plan will be finalized and approved before database lock. Major changes in statistical methodology used for the main and pre-planned analyses from this SAP, will require detailed description and justification in the statistical analysis report. The final analysis datasets, programs, and outputs are archived following good clinical practice guidelines (ICH E9).

2. Study design and objectives

2.1. Study design

This study is a multi-centre, controlled, randomized trial of 3x3 Ng/Ct screening (comparator) vs. no screening (intervention). It will be performed in the PrEP cohorts situated at the ITM, HSP, CHU, UZG and EH. All men in follow up at these five centres who report having had sex with another man in the previous year and are enrolled for PrEP follow up will be eligible to participate in the study. After signing informed consent participants will be randomized via a computer-generated schema to either 3x3 screening or no Ng/Ct screening. In both arms, participants will be followed up in an identical fashion including 3x3 screening. The only difference between the arms will be that in the screening arm, Ng/Ct results will be sent by the STI Laboratory to the

study physicians and these participants will be treated and partner contact tracing will be done. The STI Laboratory will only process the samples/report the results from the non-screening arm at the end of the study. In both arms, all individuals with symptoms compatible with Ng or Ct will be tested and treated for these infections according to current best practice guidelines. At the end of the 12-month study period, participants whose most recent tests were positive for Ng or Ct will receive treatment for these.

2.2. Study objectives

Primary objective:

To assess if not screening MSM on PrEP for Ng/Ct is non-inferior compared to screening in terms of the incidence rate of these infections over a 12-month period.

Secondary objectives:

1) To compare the antimicrobial exposure (ceftriaxone/azithromycin/doxycycline) in the screening and non-screening arms
2) To assess if not screening MSM on PrEP for Ng/Ct is non-inferior compared to screening in terms of the incidence rate of symptomatic Ng and Ct infections
3) To assess the incidence of syphilis
4) To assess the incidence of HIV
5) To evaluate the economic impact of cessation of Ng/Ct screening in MSM
6) To explore PrEP users' perceptions towards STI screening (only for ITM subjects)

3. General analysis strategy

Scheduled visits that were performed out of window (cfr. protocol) are considered as unscheduled visits, with the corresponding scheduled visit considered as 'not done'.

4. Description of study population

The study population will be described overall and in each hospital separately.

4.1. Patient accounting

Details of participants who are randomized, those who withdraw from the study after randomization and those who are lost to follow-up will be summarized in a CONSORT flow diagram. The number (%) of participants attending scheduled follow-up visits will be reported.

4.2. Description of study population

Participants in each intervention group, and in each site, will be described with respect to baseline characteristics. The description will be in terms of medians/means and quartiles/standard deviations for continuous characteristics and using counts and

percentages for categorical characteristics. The clinical importance of any imbalance will be noted though statistical tests of significance will not be undertaken.

5. Description of patient populations and outcomes

5.1. Patient populations

We will analyse the primary outcome both using Intention-to-Treat and Per-Protocol approaches, with Per-Protocol as primary approach. In the Intention-to-Treat analysis, all participants will be analysed according to their randomized allocation, even in case they receive another intervention, show protocol violations, or are lost to follow-up. In the per-protocol analysis only participants who receive intervention as planned, and follow the protocol as planned are included. For the safety analysis, all patients are included in the intervention group they actually received (all-patients-treated approach).

5.1.1. Intention to treat (ITT) analysis

In the Intention-to-Treat analysis, all participants will be analysed according to their randomized allocation, even in case they receive another intervention, show protocol violations prior to or during the study, or are lost to follow-up. The Intention-to-Treat analysis will include all randomized participants with at least one follow-up visit.

5.1.2. Per protocol (PP) analysis

In the per-protocol analysis only participants who receive intervention as planned, and follow the protocol as planned are included.
In Table 1 the protocol violations are classified as minor and major where minor violations can be included in the PP analysis population and major violations are excluded.

Table 1: The protocol violations classified as minor or major violation

Protocol Violation	Major/Minor Violation	Comments
Inclusion criteria		
1. Able and willing to provide informed consent	Major	
2. Men (born as males) and transwomen aged 18 or more	Major	
3. Has had sex with another man in the last 12 months	Major	
4. Enrolled in Belgian PrEP program at ITM/HSP/EH/CHU/UZG with approval for TDF/FTC (Tenofovir disoproxil fumarate/Emtricitabine) reimbursement from a Belgian Medical Aid	Major	
5. Willing to comply with the study procedures and to attend the clinic for the 3-monthly visits	Major	
Exclusion criteria		
1. Enrolment in another interventional trial	Major	
2. Tests HIV-positive at screening	Major	
3. Symptoms of proctitis or urethritis	Major	
Treatment violations		
1. Not following the randomized intervention (incl. participants who receive test results)	Major	
Follow-up violations		
1. Fewer than three scheduled follow-up visits with Ng/Ct result available	Major	

5.2. Efficacy study endpoints

Primary endpoint
Incidence of Ng plus Ct detected at any site.

Numerators: Cumulative number of laboratory-confirmed diagnoses of Ng plus Ct in 12 months in screening/non-screening arms. Each participant can only contribute one diagnosis of Ct and one diagnosis of Ng per (scheduled or unscheduled) visit - regardless of number of sites infected. Thus each participant can contribute up to 2 diagnoses (Ct/Ng) at each (scheduled or unscheduled) visit. The numerator includes laboratory-confirmed diagnoses made between scheduled visits, performed inside or outside of the study.

Denominators: Number of scheduled study visits with available results for the diagnosis of Ng/Ct. The denominator does not include unscheduled visits.

The diagnosis of Ng and Ct will be made via nucleic acid amplification testing (NAAT) performed on rectal and pharyngeal swabs and urine.

If the person urgently requires knowing if they are infected with Ng/Ct or not this will be done by repeat testing for these infections. If this test is positive for Ng/Ct then this will be counted as an intervisit infection in the final analysis.

In the primary analysis, the primary endpoint (as described above) includes all Ng/Ct diagnoses. Hence it is implicitly assumed that every diagnosis is a new infection. It is however possible that an Ng/Ct infection detected at the 3 to 12 month visit in the non-screening arm is simply a non-resolved infection from the prior visit. This could spuriously increase the measured incidence in the non-screening group. Because this risk only applies to the non-screening arm it results in a bias towards exaggerating the effect of screening on incidence. As such if our study finds that non-screening is non-inferior to screening this finding could not be explained by this bias. In a sensitivity analysis, the numerator of the primary endpoint will only include diagnoses if there was a negative test result at the prior visit.

Because any positive Ng/Ct test from the test of cure visits is considered a non-resolved old infection, these will not be included in the primary or secondary outcomes as new infections. If however the test of cure was to assess if an Ng infection had cleared and a new Ct infection was detected at the test of cure visit then this would be included as a new infection in the primary and secondary study outcomes.

Secondary endpoints

1) Antimicrobial exposure will be measured as number of standard doses per 1000 person-years based on standard WHO and ECDC methodology [1]. Data on all antimicrobials consumed will be collected at each study visit and will include antimicrobials consumed at other health facilities such as at participant's General Practitioner. Only start- and stop date of antimicrobial consumption is collected. It is assumed that standard treatment is received. See table below for standard treatment of STIs. If there is no information on type of Chlamydia infection, assume non-LGV. For other infections, collect information about standard treatment from investigators. The dose can then be converted to DDD standard doses based on WHO methodology [1].

Infection	Antibiotic	Dose	Duration	DDD (standard dose)
Chlamydia (LGV)	Doxycycline	100 mg 2x/day	21 days	2/day
Chlamydia (non LGV)	Doxycycline	100 mg 2x/day	7 days	2/day
Chlamydia (non LGV)	Azithromycin	1g oral	One dose	3.3

Gonorrhea	ceftriaxone + azithromycin	1g IMI +2g oral	Each one dose	0.5 ceftriaxone + 6.6 azithromycin
Syphilis	benzathine penicillin	2.4 mu (2.4mu= 1836 mg)	Either 1 or 3 doses	0.51/dose
Hepatitis C	None			

2) Incidence rate of symptomatic Ng and Ct. Similar to the primary endpoint but only taking into symptomatic infections. This information will be collected at all visits and will include episodes of symptomatic Ng and Ct diagnosed elsewhere as long as these were laboratory confirmed diagnoses – either based on molecular testing or in the case of Ng, culture.
3) Incidence rate of syphilis. The European IUSTI case definition of a syphilis infection will be followed [2].
4) Incidence of HIV
5) Economic impact of cessation of 3x3 screening in MSM in Belgium
6) Participants' perception/experience and preference for screening vs. no screening (only for ITM subjects)

6. Interim analyses

Interim analyses are planned at two time points: once 50% and 100% of all study recruits have passed their 6 month visit. For the first interim analysis, data is used of the first 507 enrolled participants (i.e. ordered by randomization date) until 6 months follow-up. For the second analysis, data is used of all enrolled participants until 6 months follow-up. The goal of the interim analyses (at both time points) is to estimate the incidence rate ratio of symptomatic Ng plus CT. Estimates will be based on a Poisson regression model (or a negative binomial regression model if there is overdispersion) with number of symptomatic diagnoses as dependent variable, arm and hospital as independent variables and log(number of visits) as offset. This model will provide estimates of the incidence rate in each arm and the incidence rate ratio (no screening versus screening), together with 95% confidence interval. Visits with missing data on the diagnosis of Ng or Ct, will be excluded from the calculation of the incidence rate (excluded both from numerator and denominator).

7. Analysis of main efficacy outcomes: Incidence of Ng plus Ct

In each arm, the incidence of Ng plus Ct will be estimated together with 95% confidence interval. Estimates will be based on a Poisson regression model (or a negative binomial regression model if there is overdispersion) with number of diagnoses as dependent variable, arm and hospital as independent variables, and log(number of visits) as offset. This model will also provide an estimate of the log incidence rate ratio (no screening versus screening), together with 95% confidence interval. The ‘no screening’ arm is proven to be non-inferior if the upper limit of the 95% confidence interval is lower than log(1.25).

Visits with missing data on the diagnosis of Ng or Ct, will be excluded from the calculation of the incidence rate (excluded both from numerator and denominator).

In a sensitivity analysis, the numerator of the primary endpoint will only include diagnoses if there was a negative test result at the prior visit.

7.1. Subgroup analyses

We will conduct a prespecified subgroup analysis to assess the incidence of Ng plus Ct in screening vs. non-screening arms in participants with lower-risk behavior. An individual with lower-risk behavior is defined a priori as those individuals who report 4 or fewer partners per 3-month period in all 5 relevant 3 month periods with data available. These periods are the 3-months prior to study enrollment as well as the four 3 month periods during study follow up. The analysis will be analogous to the analysis of the primary objective, but restricted to participants with lower-risk behavior.

7.2. Other aspects

a. Multiplicity

As this is a study with a single primary efficacy endpoint, no multiplicity adjustments are needed.

b. Missing data

Visits with missing data on the diagnosis of Ng or Ct, will be excluded from the calculation of the incidence rate (excluded both from numerator and denominator).

8. Analysis of secondary objectives

8.1. To compare the antimicrobial exposure (ceftriaxone/azithromycin/doxycycline) in the screening and non-screening arms

A Poisson regression model model (or a negative binomial regression model if there is overdispersion) with number of standard doses as dependent variable, arm and hospital as independent variables and log(time at risk) as offset will be fitted. Time at risk will be calculated from day of enrollment until end of follow-up.

8.2. To assess if not screening MSM on PrEP for Ng/Ct is non-inferior compared to screening in terms of the incidence rate of symptomatic Ng and Ct infections

In each arm, the incidence of symptomatic Ng plus Ct will be estimated together with 95% confidence interval. Estimates will be based on a Poisson regression model (or a negative binomial regression model if there is overdispersion) with number of symptomatic diagnoses as dependent variable, arm and hospital as independent variables and log(number of visits) as offset. This model will also provide an estimate of the log incidence rate ratio (no screening versus screening), together with 95% confidence interval. The ‘no screening’ arm is proven to be non-inferior if the upper limit of the 95% confidence interval is lower than log(1.25).

Visits with missing data on the diagnosis of Ng or Ct, will be excluded from the calculation of the incidence rate (excluded both from numerator and denominator).

8.3. To assess the incidence of syphilis

For this analysis, we only consider the first syphilis infection for each participant. A Poisson regression model (or a negative binomial regression model if there is overdispersion) with incident syphilis infection as dependent variable, arm and hospital as independent variables and log(time at risk) as offset will be fitted. Time at risk will be calculated from day of enrollment until end of follow-up or date of first syphilis infection. The incidence rate of syphilis with 95% confidence interval in each arm will be estimated from this model.

8.4. To assess the incidence of HIV

A Poisson regression model (or a negative binomial regression model if there is overdispersion) with incident HIV infection as dependent variable, arm and hospital as independent variables and log(time at risk) as offset will be fitted. Time at risk will be calculated from day of enrollment until end of follow-up or date of HIV infection.

8.5. To evaluate the economic impact of cessation of Ng/Ct screening in MSM

This analysis will be performed by the health economist.

8.6. To explore PrEP users' perceptions towards STI screening (only for ITM subjects)

This analysis will be performed by the social scientist.

9. Safety analyses

The number and proportion of participants who die in each intervention group will be presented.

10. References

[1] "ATC/DDD Index 2021." [Online]. Available: https://www.whocc.no/atc_ddd_index/.

[2] M. Janier *et al.*, "2014 European guideline on the management of syphilis," *J. Eur. Acad. Dermatology Venereol.*, vol. 28, no. 12, pp. 1581–1593, Dec. 2014.