NOTTINGHAM CLINICAL TRIALS UNIT

The G-T G Trial

Gentamicin in the treatment of gonorrhoea (G-ToG)

Statistical Analysis Plan

Final version 1.0
(22 Feb 2017)

Based on Protocol version 2.0 (dated 17 June 2015)

Trial registration ISRCTN51783227

The following people have reviewed the Statistical Analysis Plan and are in agreement with the contents			
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Amendment to versions

Protocol version	Updated SAP version no.	Description of and reason for change	Date changed
Version 2.0	Version 0.2	Following comments from Trish	01 Apr 2016
Version 2.0	Version 0.3	Following comments from Jonathan	05 Apr 2016
Version 2.0	Version 0.4	Following comments from Mike Bradburn	25 Apr 2016
Version 2.0	Version 0.5	Following discussion with Jonathan to add additional exploratory analyses	12 Oct 2016
Version 2.0	Version 0.6	Added further assumptions on sample collection and deriving primary outcome Primary analysis and binary secondary outcome analyses modelled updated to GEE	06 Feb 2017
Version 2.0	Version 1.0	Following further comments from Mike, added more details about estimating risk difference using GEE model, added N(%) of participants experiencing side effects and adverse events.	22 Feb 2017

Justification of change of primary outcome analysis model

Protocol	**Amendment**	**Rationale**
Analysis of primary outcome: The evaluation of the primary clinical outcome variable will be performed using a general linear model for binary outcome adjusted by clinic site. The primary efficacy parameter comparing gentamicin with ceftriaxone will be the risk difference in the proportion of participants clear of infection at two weeks follow up along with the 95% confidence interval. Gentamicin will be regarded as non-inferior if the lower 95% confidence limit for the risk difference in confirmed clearance is -5 percentage points or greater.	On 17-Jun-2015 and 03-Jun-2015 the trial team decided to include more centres to boost recruitment. The primary analysis will now be performed as follows: The primary approach to between-group comparative analyses will be by intention-to-treat without imputation of missing outcome data for clearance of gonorrhoea at 2 weeks. The evaluation of the proportion of participants with clearance of gonorrhoea at 2 weeks will be performed using a generalised estimating equations (GEE) for binary outcomes adjusted by recruiting centre as a random effect with robust standard errors.	Justification of the change of primary outcome analysis model: the introduction of centres recruiting smaller numbers of participants made it possible that there could be centres where there were no treatment failures. Therefore the original model/analysis method was no longer appropriate. To account for some centres without any treatment failures GEE model is deemed more appropriate as it adjusts centre as random effect rather than fixed effect.

Abbreviations

Abbreviation	Description
AC	Aptima Combo
AE	Adverse Event
BD	Becton Dickinson
DMC	Data Monitoring Committee
eGFR	Estimated glomerular filtration rate
GEE	Generalised estimating equations
HIV	Human immunodeficiency virus
ITT	Intention to treat
MIC	Minimal inhibitory concentration
MSM	Man having sex with man
NAAT	Nucleic acid amplification test
SAP	Statistical Analysis Plan
SD	Standard deviation
STI	Sexually Transmitted Infection
TMG	Trial management group
TSC	Trial Steering Committee
VAS	Visual Analogue Scale

INTRODUCTION & PURPOSE

This document details the rules proposed and the presentation that will be followed, as closely as possible, when analysing and reporting the main results from the HTA funded G-TOG trial.

The purpose of the plan is to:

1. Ensure that the analysis is appropriate for the aims of the trial, reflects good statistical practice, and that interpretation of a priori and post hoc analyses respectively is appropriate.

2. Explain in detail how the data will be handled and analysed to enable others to perform or reproduce the actual analysis.

Additional exploratory or auxiliary analyses of data not specified in the protocol, but planned prior to database lock will be included in this SAP highlighting their purpose. The results of such explorations will not be revealed prior to interpretation of the main trial results. Any additional analyses requested after database lock will be considered and if conducted, will be identified as post hoc exploratory analyses when they are reported.

The analysis plan will be made available if required by journal editors or referees when the main papers are submitted for publication.

Amendments to the statistical analysis detailed in this plan will be described and justified in the final report of the trial.

1. SYNOPSIS OF STUDY DESIGN AND PROCEDURES

1.1. Trial aims and objectives

The purpose of the trial is to determine the effectiveness and safety of gentamicin in the treatment of gonorrhoea.

1.1.1. Primary objective

The primary objective of the study is to determine whether gentamicin is an acceptable alternative to ceftriaxone, in the treatment of gonorrhoea. This will be addressed by determining whether the rate of microbiological clearance of Neisseria gonorrhoeae in participants treated with gentamicin is non-inferior to the clearance in participants treated with ceftriaxone.

1.1.2. Secondary objectives

Secondary objectives of the study are:

- To determine whether a single intramuscular dose of gentamicin is safe and well tolerated
- To determine whether a single intramuscular dose of gentamicin is cost effective to the NHS when used to treat gonorrhoea
- To determine the relationship between clinical effectiveness and the laboratory measurement of antibiotic effectiveness (the minimum inhibitory concentration (MIC) required to inhibit growth of *N. gonorrhoeae*)

1.2. Trial design and configuration

G-TOG is phase III, multi-centre, parallel group, investigator-blinded, non-inferiority randomised controlled trial.

1.3. Trial centres

The following centres are included in the study for recruitment:

- Birmingham
- Barts
- Guy's and St Thomas'
- Leeds
- Manchester
- Sheffield
- Southampton
- Chelsea and Westminster
- Brighton
- Coventry
- Royal Free
- Royal Berkshire
- St Mary's
- John Hunter Clinic
- Middlesex

1.4. Eligibility criteria

1.4.1. Inclusion criteria

Individuals must meet ALL of the following to be included in the study

- Individuals aged 16-70 years.
- Diagnosis of uncomplicated untreated* genital, pharyngeal or rectal gonorrhoea based on a positive gram stained smear on microscopy, or positive NAAT within the last 4 weeks.
- Written informed consent provided.

*patient has not received any antibiotic in previous 28 days which could have treated gonorrhoea (either partially or completely).

1.4.2. Exclusion criteria

Individuals will be excluded from the study if they meet ANY of the following

- Known concurrent bacterial sexually transmitted infection (apart from chlamydia).
- Known bacterial vaginosis or Trichomonas vaginalis
- Known contra-indications or allergy to gentamicin, ceftriaxone, azithromycin or lidocaine.
- Pregnant or breast-feeding.
- Current clinical diagnosis of complicated gonorrhoea infections eg pelvic inflammatory disease, epididymo-orchitis.
- Weight less than 40kg at the time of randomisation
- Currently receiving or have received ceftriaxone, gentamicin or azithromycin within the preceding 28 days.
- Previous participation in this study

1.5. Description of interventions

There are two treatment arms within the study:

- Gentamicin (240mg) administered as a single intramuscular injection.
- Ceftriaxone (500mg) administered as a single intramuscular injection.

Both groups will also receive azithromycin (1g) administered orally.

1.6. Randomisation procedures

Participants will be assigned to treatment groups using a remote internet based randomisation system maintained by the Nottingham Clinical Trials Unit (NCTU). Access to the system will be granted by the NCTU in accordance with the responsibilities on the delegation log.

The randomisation schedule is based on a computer generated pseudo-random code using random permuted blocks of randomly varying size, created by the Nottingham Clinical Trials Unit (NCTU) in accordance with their standard operating procedure (SOP) and held on a secure University of Nottingham server. The randomisation will be stratified by clinical centre.

1.7. Sample size and justification

Based on an efficacy rate of 96% for the ceftriaxone regimen which is consistent with previous trials, a total sample size of 646 for analysis (323 in each group) will achieve 90% power to detect non-inferiority with lower confidence limit for the absolute risk difference of 5%. The significance level is 0.025. To allow for a loss to follow-up rate of up to 10%, the study will recruit a total of 720 participants. A 5% lower confidence interval was acceptable to 17 of 24 sexual health patients whose opinion was sought, and provides a realistic recruitment target.

1.8. Blinding and breaking of blind

Only the nurse/doctor randomising and administering the treatment will know what treatment the participant has been randomised to. Members of the research team who are aware of the treatment allocation will not have any role in data collection. The participant and staff involved in the care and assessment of the participant will not know what treatment they have been randomised to. This should ensure the minimisation of any bias in assessment due to knowledge of the treatment administered.

To maintain the overall quality and legitimacy of the clinical trial, blind break should only occur in exceptional circumstances when knowledge of the actual treatment is absolutely essential for further management of the patient. Investigators are encouraged to discuss with the Chief Investigator if he/she believes that unblinding is necessary.

It is unlikely that individual treatment allocations will have to be prematurely disclosed since the intervention is a single dose and there is no antidote to the intervention. However, there will be an electronic web-based system available 24 hours a day where authorised personnel are able to log in to determine what treatment a participant has received. This system will record who has broken the blind, when and for what reason. If emergency unblinding is deemed to be necessary, this system should be used.

The Investigator is encouraged to maintain the blind as far as possible.

1.9. Trial committees

A number of committees were assembled to ensure the proper management and conduct of the trial, and to uphold the safety and well-being of participants. The general purpose, responsibilities and structures of the committees were described in the protocol. However each committee developed its own rules and procedures which may evolve with time.

Trial Management Group: The Trial Management Group (TMG) oversaw the operational aspects of the trial. The TMG met regularly to review the progress of the trial and addressed any issues arising.

Trial Steering Committee: The Trial Steering Committee (TSC) was set up with an independent Chairperson and monitored, reviewed and supervised the progress of the trial. The independent Trial Steering Committee monitored blinded data to consider safety and efficacy indications. The TSC may recommend discontinuation of the study if significant ethical or safety concerns arose or if there was very clear evidence of benefit (clinical or statistical) prior to completion of the study. The TSC considered reports from the DMC when making recommendations.

The TSC met independently prior to the start of the study and agreed terms of reference.

Data Monitoring Committee: An independent Data Monitoring Committee was established with access to unblinded data to provide independent review and recommendation in the light of potential treatment effect. The DMC met or teleconference prior to the start of the study and

agreed terms of reference and a provisional meeting or teleconferencing schedule. Only the Data Monitoring Committee had access to unblinded data until the final outcome assessment was completed.

1.10. Outcome measures

1.10.1. Primary outcome

The primary outcome measure is clearance of N. gonorrhoeae at all infected sites confirmed by a negative NAAT, two weeks post treatment (as recommended by the British Association for Sexual Health and HIV). The results from the AC NAAT will be considered primary.
Infection sites include genital, rectal and pharyngeal sites. Note that urine and urethra are interchangeable genital samples and as are vagina and cervix.

1.10.2. Secondary outcomes

Secondary outcomes are

- clinical resolution of symptoms
- frequency of nausea/vomiting, hearing loss, dizziness and rash.
- frequency of other adverse events
- tolerability of therapy
- relationship between clinical effectiveness and MIC to inhibit N. gonorrhoeae
- cost effectiveness

Note a separate analysis plan will contain details of analysis approaches for cost effectiveness.

1.11. Interim analysis

No formal interim analyses are planned for this trial.

2. GENERAL ANALYSIS CONSIDERATIONS

2.1. General considerations

All summaries and statistical analyses will be conducted using Stata Version 13.1 or above according to this analysis plan. Unless otherwise stated, analyses of efficacy parameters will be performed on the ITT set, and analyses of safety parameters will be performed on the safety set. The number of participants included in each summary/analyses will be presented on tables and where appropriate the number of participants with missing data will be presented. Minimums and maximums will be presented to the same number of decimal places as the raw data, and means, medians (where appropriate) and SDs will be presented to one more decimal place than the raw data, percentages will be rounded to the nearest whole number.

2.2. Analysis populations

ITT set: Participants as randomised regardless of the adherence with their allocated group and without imputation for missing data (intention to treat principle (ITT)).

Safety set: Participants as per their received treatment.

No specific per protocol set will be defined as several sensitivity analyses will be performed on the primary outcome.

2.3. Derived variables

Clearance of gonorrhoea is tested by AC NAAT which is an automated laboratory test and therefore it is not subject to bias through knowledge of treatment allocation. The method of NAAT performed varies between centres. The AC NAAT test will be considered gold standard. Therefore for those centres where the AC NAAT method is not used by the local laboratory; additional swabs will be taken and sent for testing at a central laboratory using this method. This will ensure consistency in diagnosis and response for all participants for ascertaining the primary end point of the study. Only local lab tested positive samples at baseline will be re-tested at follow up for determining clearance. Genital, pharyngeal and rectal samples are required for females and MSMs (men having sex with men) and genital samples only are required for heterosexual men.

Resolution of each individual clinical symptom will be defined as absence of the symptom at 2 weeks which was present at baseline.

In addition to summarising the absolute change in creatinine from baseline to 2 weeks for each participant the following variables will be derived:

1) Whether or not participant met clinically important change from baseline to 2 weeks (defined as an increase or decrease of more than 30% from baseline).
2) Whether or not creatinine level at 2 weeks exceeds upper limit of normal value. These upper limits are centre specific and calculations will be done based on local lab values at each centre.
3) Change in eGFR from baseline at 2 weeks, eGFR is calculated from the CKD-EPI equation[1] which uses creatinine.

2.4. Procedures for missing data

The primary analysis will be performed on the ITT set without imputation of missing data for clearance of gonorrhoea at 2 weeks.
The results of positive pre-trial tests will be used where the baseline tests do not show any sites positive for gonorrhoea or are missing.

Sensitivity analyses of the primary outcome will be performed on the ITT set to check the robustness of the conclusions to missing outcome data. The pattern of missing data will be explored overall and in each of the two treatment groups. Where clearance at 2 weeks using AC NAAT is missing but there is data for BD NAAT, the results of the BD NAAT will be used for the sensitivity analysis.

Three imputation methods will then be applied when data for the clearance of gonorrhoea (both AC and BD NAAT) at 2 weeks is missing:

- Multiple imputation using chained equations
- Assume all missing data as cleared of gonorrhoea
- Assume all missing data as not cleared of gonorrhoea

The table below outlines possible scenarios of primary outcome being available, with all others being missing.

Baseline Visit		Follow-up Visit		Primary outcome available?
Tests	**Results**	**Tests**	**Results**	
All required samples taken	>=1 positive	All positive re-tested	Any	**Yes**
	No positive*	All pre-trial positive re-tested	Any	**Yes**
Not all required samples taken	>=1 positive	All positive (from pre-trial and baseline) re-tested	Any	**Yes**
	No positive	All pre-trial positive re-tested	Any	**Yes**
Any follow up NAAT AC test shown positive				**Yes**

**Based on baseline NAAT test and Gram Stain test.*

3. DESCRIPTION OF PARTICIPANT CHARACTERISTICS

3.1. Participant flow

Details about the numbers screened, excluded (with reasons), recruited and followed up will be summarised in a CONSORT flow diagram.

3.2. Baseline characteristics

Continuous variables at baseline will be summarised for each treatment arm, in terms of the mean, standard deviation, median, lower and upper quartiles, minimum, maximum and number of observations. Categorical variables will be summarised in terms of frequency counts and percentages. These baseline variables include age, gender, ethnicity, country of birth, medical history, STI history, creatinine level, BMI, antibiotic use and number and name of infected sites.

4. ASSESSMENT OF STUDY QUALITY

4.1. Randomisation

Number of participants recruited will be tabulated by recruiting centre and treatment arm

4.2. Adherence

Any instance of non-adherence will be listed alongside treatment arm and reason for non-adherence.

4.3. Protocol deviations

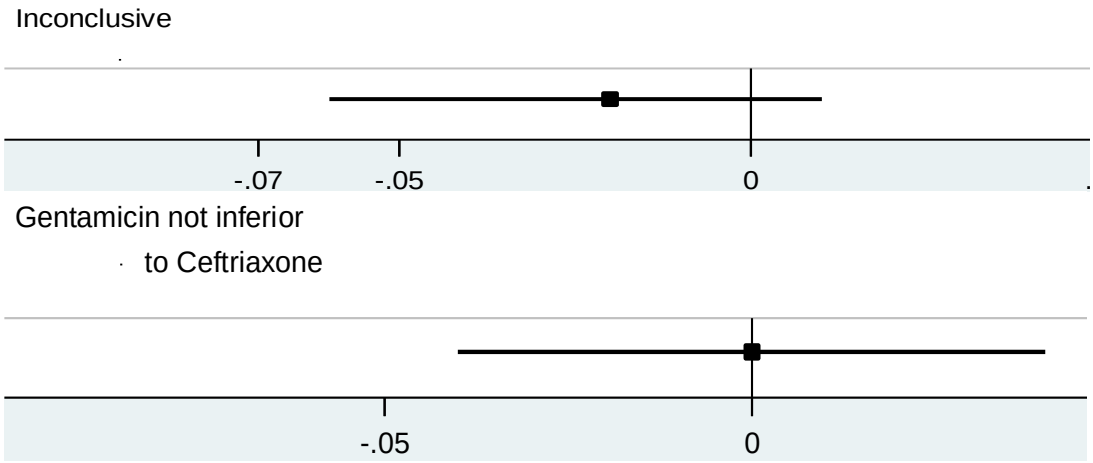
All protocol deviations captured on the database will be summarised and listed by treatment arm.

5. ANALYSIS OF EFFECTIVENESS/EFFICACY

5.1. Primary analysis

The primary approach to between-group comparative analyses will be by intention-to-treat without imputation of missing outcome data for clearance of gonorrhoea at 2 weeks. The evaluation of the proportion of participants with clearance of gonorrhoea at 2 weeks will be performed using a generalised estimating equations (GEE) for binary outcomes adjusted by recruiting centre as a

random effect. The GEE model will be using identity link function to enable estimation of adjusted risk difference. The primary efficacy parameter comparing gentamicin with ceftriaxone will be the risk difference in the proportion of participants clear of infection at all sites by AC NAAT at two week follow up along with the 95% confidence interval. Gentamicin will be regarded as non-inferior if the lower 95% confidence limit for the risk difference (Gentamicin group versus Ceftriaxone group) in confirmed clearance is -5 percentage points or greater. The graph below shows different scenarios of conclusions based on the estimates and confidence intervals.



5.2. Sensitivity analysis of primary outcome

In addition to the sensitivity analyses outlined in section 2.4 we will also investigate the treatment efficacy by performing the following sensitivity analyses:

1) Exclude participants who did not have any positive samples at baseline
2) Exclude participants who did not received allocated treatment
3) Exclude participants who did not have full baseline samples taken, i.e. females and MSMs (men having sex with men) should have genital, rectal and pharyngeal samples taken; heterosexual men should have genital samples taken.
4) Further adjust baseline variables with marked imbalance between arms

The results of these sensitivity analyses should be considered supportive to the primary analysis.

5.3. Secondary outcomes

Clearance of gonorrhoea by infection site:
Participants may have infection at multiple sites, and up to seven different combinations of 1/2/3 sites are possible. For each of the three infection sites, we will separately estimate clearance by treatment arm along with 95% confidence intervals, rather than formally fit an interaction term for different combinations of infection site in the regression model. Any suggestion of a differential effect according to infected site would require confirmation in future research.

Clinical resolution of symptoms:
The evaluation of clinical resolution will be performed using generalised estimating equations (GEE) for binary outcome adjusted by recruiting centre as random effect. The efficacy parameter

comparing gentamicin with ceftriaxone will be the risk difference in the proportion of participants clear of clinical symptoms at two week follow up along with the 95% confidence interval.
These symptoms are genital discharge, dysuria, sore throat, ano-rectal pain, rectal bleeding, rectal discharge, tenesmus, constipation, intermenstrual bleeding, post-coital bleeding.

Creatinine level at 2 weeks:
The creatinine related binary outcomes and change in eGFR will be summarised using basic descriptive statistics. Shift plots will also be presented to identify extreme values.

Minimal Inhibitory Concentration (MIC) for trial medications:
The MIC data will be summarised overall and by infection site separately for gentamicin, ceftriaxone and azithromycin. It is expected that some data values will be below or above quantifiable limits therefore plots of the MIC value distribution for each medication (Ceftriaxone, Gentamicin and Azithromycin) will be produced. This data will also be presented by treatment arm and infection site.

Concomitant medications
Antibiotics and other concomitant medications taken during the trial will be listed by treatment group.

NHS health service use
Descriptive summaries of health service use during the trial will be provided by treatment group, in terms of:

- Number of GP appointments
- Number of consultations with a doctor at a sexual health clinic
- Number of A&E department visits
- Hospital admission related to diagnosis of gonorrhoea
- Whether or not additional medication prescribed for gonorrhoea

6. ANALYSIS OF SAFETY

6.1. Side effects/Adverse events

Descriptive summaries of side effects and adverse events by treatment group will be provided, in terms of:

- Number and percentage of participants who reported each of the following: nausea, vomiting, hearing loss, dizziness, and rash. The total incidences of each of the side effects will also be summarised.
- Severity and time in hours/days from injection to onset of each of the following: nausea, vomiting, hearing loss, dizziness and rash.
- VAS pain score following injection,
- Listings of all non-serious AEs and all SAEs, which will be coded using MedDRA coding dictionary version 17.1. Number and proportion of participants who experienced any AE or SAE will also be summarised.

7. EXPLORATORY ANALYSES

The following analyses will be performed on an exploratory basis after the results for the primary and secondary outcomes have been interpreted. They are not expected to form part of the main trial reports.

1. Association between injection pain (VAS) and the following variables: injecting nurse, participant age and actual treatment received.
2. Prevalence of rectal or pharyngeal gonorrhoea in those with no history of anal of oral sex
3. Relationship between participants who had no positive samples at baseline and those who reported any antibiotic use before trial entry or previous history of gonorrhoea
4. Number and proportion of those with positive chlamydia at baseline and negative at 2 weeks, by site of infection
5. Number and proportion of NAAT tests in vaginal and cervical at both baseline and follow up tests in women
6. Time taken for symptoms (genital discharge, dysuria) to resolve after treatment, and its potential predictors: previous gonorrhoea, co-infection with CT, antibiotic given. This exploration will exclude those with sexual contact with new/untreated partner after treatment.
7. Average time for patients with symptoms to present at clinic and its potential predictors: age, gender, ethnicity, site of infection and severity of symptoms
8. Frequency of symptoms in pharyngeal and rectal infections in those with baseline NAAT positive
9. Frequency of sexual contact in the 2 weeks after treatment in terms of: use of condoms, number of new partners, number of existing partners and frequency of sexual contact.
10. Frequency of urethral symptoms in men who have sex with men (MSM) prior to receiving the treatment
11. Number and proportion of pharyngeal infections which were positive at 2 weeks

8. REFERENCE

1. www.ckdepi.org/equations/gfr-calculator/