IMPRESU – WP3
Statistical Analysis Plan (SAP)

The effect of methenamine hippurate to reduce antibiotic prescribing due to new episodes of urinary tract infections (UTI) in elderly women with recurrent UTI – a triple-blinded, randomized placebo-controlled phase IV study

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TABLE OF CONTENTS

1. INTRODUCTION

1.1. Background

Recurrent urinary tract infection (UTI) in elderly women is a major driver of antibiotic prescription (1-9). Hence, the question of feasible and appropriate preventive measures are important issues in this field. Methenamine hippurate is frequently prescribed in Norway and Sweden as prophylaxis for recurrent UTI (9). Methenamine hippurate is hardly used outside of Scandinavia. Methenamine hippurate acts via the production of formaldehyde from hexamine, which in turn acts as a bacteriostatic agent, therefore methenamine hippurate is not defined as an antibiotic. According to a Cochrane review 2012 the rates of adverse events for preventing UTI was low (10). Although this review showed methenamine hippurate might be effective in preventing UTI in the short term, there is a need for large well-conducted randomised controlled trial (RCT) to clarify both the safety and effectivity of preventive methenamine hippurate for longer term use (11-16). This is particularly important for longer term use for people without neuropathic bladder disorders (10). A Norwegian longitudinal observational study including women aged 50-80 years with recurrent UTI indicated a significant and large reduction of more than 50% in antibiotic prescriptions for UTI after start of prophylactic methenamine hippurate (Gjelstad, personal communication). This further strengthens the need for an RCT of methenamine hippurate as prophylaxis for recurrent UTI.

Escherichia coli (E. coli) is a part of the human gastrointestinal microbiota (17). Uropathogenic E.coli from fecal reservoirs are the predominant causative microbes in uncomplicated UTIs (18-20). Previous studies have shown that strains of E.coli can be divided into phylogenetic subgroups (A, B1, B2, C, D, E and F), and that subgroup B2 and D represents the most virulent types and are associated with extra-intestinal infections (21-23).
We aim to investigate whether the phylogenetic subgroups of E.coli present in the urine cultures at inclusion can have a modifying role on the preventive effect of Methenamine hippurate in this study. If feasible, we will also investigate whether or not subsequent episodes of acute UTIs in elderly women with recurrent UTI are caused by E.coli of the same phylotype.

1.2. Rationale for conducting this study

Existing knowledge suggests that methenamine hippurate is a safe drug with few and mild side effects and with the potential to significantly reduce antibiotic usage for women with recurrent UTIs. Methenamine hippurate has been on the market for a long time but has never been tested to prevent recurrent UTIs in larger RCTs with long time follow-up. Hence, this must be proven in a large randomised trial before recommending large scale use of this drug (10).

2. STUDY OBJECTIVES AND ENDPOINTS

The primary objective of this study is to investigate if taking methenamine hippurate reduce the need for antibiotic usage due to recurrent UTI (measured as number of antibiotic courses). The remaining objectives are considered secondary. Pyelonephritis, hospitalization and death will be registered as safety endpoints in the study.

Objectives		Outcome Measures / variables / endpoints	Time point(s) of evaluation of this outcome measure (if applicable)
Primary Objective			
1	The primary objective of this study is to investigate if taking methenamine hippurate reduces the need for antibiotic usage due to recurrent UTI (measured as number of antibiotic courses).	Number of UTI antibiotic treatments during the six months of treatment. If the participant receives >1 antibiotic course for UTI without symptom relief it is regarded as one episode and counted as one antibiotic treatment. If there has been an asymptomatic period of at least 14 days in-between two UTI antibiotic courses, this is regarded as a new antibiotic treatment.	After six months of treatment.
Secondary Objectives			
2a	To investigate if methenamine hippurate will have a prolonged effect on antibiotic usage even after discontinuation.	Number of UTI antibiotic treatments during the six months following completion of treatment. If the participant receives >1 antibiotic course for UTI without symptom relief it is regarded as one episode and counted as one antibiotic treatment. If there has been an asymptomatic period of at least 14 days in-between two UTI antibiotic courses, this is regarded as a new antibiotic treatment.	Six months after completing (12 months after commencing) treatment.
2b	To investigate if taking methenamine hippurate reduces the incidence of UTI.	Number of UTIs (acute symptoms specific/related to the urinary tract) during the six months of treatment. If the participant has had >1 UTI episode without symptom relief it is regarded as one episode. If there has been an asymptomatic period of at least 14 days in-between two UTI episodes, this is regarded as a new episode.	After six months of treatment.
2c	To investigate if methenamine hippurate can reduce severity of UTI symptoms.	Registration of symptom severity when initiating treatment for UTI.	After six months of treatment.
2d	To investigate if methenamine hippurate can reduce duration of UTI episodes.	Registration of number of days of symptoms during UTI episodes.	After six months of treatment.

2e	To investigate if number of complications such as pyelonephritis and hospital admission for UTI differ between methenamine hippurate and placebo.	Registration of number of pyelonephritis and hospital admission for UTI.	Six and 12 months after commencing treatment.
2f	To investigate if phylogenetic subgroups of E. coli found at inclusion is an effect modifier in the above outcomes.	(see above)	(see above)

3. STUDY DESIGN AND PROCEDURES

3.1. Overall study design and flow chart

Study design: Blinded randomised controlled phase IV trial where patients are randomised to active intervention (methenamine hippurate) or controls (placebo).
Setting and study population: Women aged ≥70 years with recurrent UTIs in primary care. A total of 400 patients will be randomized in this trial, with approximately 100 patients in each of the participating countries; Norway, Sweden, Poland and the Netherlands.

Diagram 1. Schematic diagram of procedures:

Visit/ Telephone contact	Visit 1 Screening and randomization (®)	Telephone follow-up in case of acute UTI episode(s)	End of treatment	End of study
Day(s)	1	2-360	180	360
Methenamine hippurate (N=200) Placebo (N=200)	R			

3.2. Description of study visits

Day(s)	Visit 1 Screening and randomisation 1	Telephone follow-up in case of acute UTI episode(s) 2-360	Telephone follow-up every 30 days in all patients 2-180	Telephone contact by end of treatment 180	Telephone contact by end of study 360
Informed consent	X				
Demography, level of care	X				
Medical history	X				
Physical examination,	X				
Dipstick urinalysis and urine culture	X	X			
Inclusion/exclusion criteria	X				
Randomisation	X				
Patient Reported Outcome		X	X	X	X
Concomitant medication**	X				
Serious Adverse Events		X	X	X	X*

* In case of SAE present and not resolved by day 360, this will be followed until resolution.
** After visit 1 concomitant medication will only be registered in case of SAE, otherwise not.

4. STUDY MEASUREMENTS AND VARIABLES

- number of UTI antibiotic treatments (name of drug, dosage and duration) during day 2-360
- number of UTIs (acute symptoms specific/related to the urinary tract) during day 2-360
- registration of symptoms and severity when initiating treatment for UTI. This will be measured by a scale 0-6 (no symptoms to worst possible) for each of the three cardinal symptoms and general condition.
- registration of number of pyelonephritis and hospital admission for UTI
- laboratory tests at Visit 1: urine culture and dipstick urinalysis regarding pH, nitrite and leukocyte esterase
- laboratory tests ordered by the ordinary clinician in case of episodes of UTI during the study period (urinary dipsticks regarding nitrite and leukocyte esterase and urine culture)
- level of care: general practice, residential home care or nursing home
- SAEs, whereas relationship with IMP cannot be excluded.

4.1. Primary variable

(See section “2 Study Objectives and Endpoints”).

4.2. Secondary variable(s)

(See section “2 Study Objectives and Endpoints”).

4.3. Safety variable(s)

Pyelonephritis, hospitalization and death will be registered as safety endpoints. Methenamine hippurate has a well-documented safety profile and is a commonly used medication in primary care settings in Scandinavia. Therefore, non-serious adverse events (AE) will not be recorded in this study. All SAEs, whereas relationship with IMP cannot be excluded, occurring during the study period are recorded and reported according to guidelines in each country.

5. STATISTICS

5.1. Statistical analysis

It is the intention to adjust all analysis for the following confounding variables obtained at visit 1

- patient's age
- urine acidity (pH)
- number of antibiotic courses for UTIs in the 12 months preceding inclusion in the study
- presence of urease/stone-producing bacteria in urine culture day 1 such as proteus, some klebsiella species, Morganella morganii, Corynebacterium urealyticum and providencia
- use of local oestrogen
- patient has diabetes mellitus
- obesity defines as BMI ≥30
- presence of known mild abnormality of the urogenital tract
- if the patient is sexually active.
- If the patient previously have experienced urinary tract stone

Phylogenetic subtype of pure cultures of E.coli in the urine culture at inclusion will be analysed in a separate analysis if the number of cultures available for typing are not too small.

The statistical calculation relates to the study objectives described in section "2 Study Objectives and Endpoints":

1 Negative binomial multivariable regression will be used where number of UTI antibiotic treatments will be the dependent variable. Group allocation together with the confounding variables above will be independent variables. Histograms of the dependent variable will determine if we need to adjust for zero inflation (or any other inflation).

2a Will be analysed using the same statistical approach as objective 1 above.

2b Will be analysed using the same statistical approach as objective 1 above. Number of UTIs will be the dependent variable.

2c The outcome variable is measured at first day of a UTI using a six grade ordinal scale. The median value from all UTIs will be used if the patient has more than one episode of UTI. Data will be analysed using ordinal multivariable logistic regression.

2d Will be analysed using the same statistical approach as objective 1 above. Number of days with symptoms will be the dependent variable. The median value from all UTIs will be used if the patient has more than one episode of UTI.

2e Will be analysed using the same statistical approach as objective 1 above. Number of severe events such as pyelonephritis or hospital admission will be the dependent variable.

2f Phylogenetic subgroup of pure cultures of E.coli in the inclusion urine culture will be analysed in a separate statistical analysis as an independent variable We will add also an interaction term between phylogenetic subgroup and group allocation. All statistical analyses above will

be redone adding phylotype and the interaction term as extra independent variables. (skriv om indelning av fylotyper). Only a limited number of independent variables will be included due to the lower number of observations. These will be determined by choosing the most statistically significant independent variables used in the prior regressions. The minimal model allowed will contain the following independent variables: group allocation, phylogenetic subgroup and their interaction term. The latter require at least 30 observations. A fourth independent variable will be added if there are at least 40 observations and so on.

5.1.1. Intention to treat analysis and missing data

The primary statistical analysis is intention to treat including all patients randomized contributing with a full set of baseline observations. A sensitivity analysis will be done also including patients randomized with incomplete baseline observations where we may have to omit one or a few baseline covariates from the regression analyses.
Patients leaving the study completing at least three but less than six of the first six months will contribute with data up until they leave the study and this data will be extrapolated as if it was six months.. If they remained for less than three months their observations will be imputed with the mean from similar patients. Similar patients are defined as having an age of ± five years and belonging to the same intervention group. Patients leaving during the second six months period will also contribute with data as described above.

5.1.2. Complete case analysis

A complete case analysis will also be made where only randomised patients having a complete data set will be included.

5.1.3. Per protocol analysis

A per protocol analysis including patients consuming at least 80% of dispensed study medication will be made.

6. REASONS FOR CHANGE FROM ORIGINAL PLANNING

During discussions within the research team we have decided to make some changes to the original statistical analysis plan described in the published study protocol:

- We changed the choice of regression model from standard linear regression to negative binomial regression since the dependent variable is an integer. However, all objectives and choice of independent variables remain unchanged.
- For objective 2c the number of scale steps is only 6 and we decided ordinal regression is a better method than standard linear regression on rank transformed data.
- The statistical analysis of objective 2f was previously described as being done if we had at least 60 observations. In further discussions we decided to clarify this as including as many independent variables as possible if the condition 10 observations for each independent variable is fulfilled. This gives a structure for how to choose independent variables.
- Imputation of data in intention to treat analysis was changed for patients dropping out after participating less than 3 months. We believe it would be a better representation to use the

average from similar patients remaining in the study rather than extrapolating data from a very short time frame.

- In the original study protocol (version 11.1) we first planned an intention to treat analysis as the main analysis, a complete case analysis as a secondary analysis and that a per protocol analysis would not be made. When the study protocol was published we decided to add that a per protocol analysis will also be made (26).

All decisions above were taken before data collection were completed and before any data were analysed.

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8. APPENDICIES

Appendix 1 - Study protocol version 11.1
Appendix 2 - Publication of study protocol