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# Short course antibiotic treatment of Gram-negative bacteremia (GNB5): A study protocol for a randomized controlled trial

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## ABSTRACT

**Introduction:** Prolonged use of antibiotics is closely related to antibiotic-associated infections, antimicrobial resistance, and adverse drug events. The optimal duration of antibiotic treatment for Gram-negative bacteremia (GNB) with a urinary tract source of infection is poorly defined.

**Methods and analysis:** Investigator initiated multicenter, non-blinded, non-inferiority randomized controlled trial with two parallel treatment arms. One arm will receive shortened antibiotic treatment of five days and the other arm will receive antibiotic treatment of seven days or longer. Randomization will occur in equal proportion (1:1) no later than day five of effective antibiotic treatment as determined by antibiogram. Immunosuppressed patients and those with GNB due to non-fermenting bacilli (Acinetobacter spp., Pseudomonas spp.), Brucella spp., Fusobacterium spp. or polymicrobial growth are ineligible.

The primary endpoint is 90-day survival without clinical or microbiological failure to treatment. Secondary endpoints include all-cause mortality, total duration of antibiotic treatment, hospital

readmission, and *Clostrioides difficile* infection. Interim safety analysis will be performed after the recruitment of every 100 patients. Given an event rate of 12%, a non-inferiority margin of 10%, and 90% power, the required sample size to determine non-inferiority is 380 patients. Analyses will be performed on both intention-to-treat and per-protocol populations.

**Ethics and dissemination:** The study is approved by the Danish Regional Committee on Health Research (H-19085920) and the Danish Medicines Agency (2019-003282-17). The results of the main trial and each of the secondary endpoints will be submitted for publication in a peer-reviewed journal.

**Trial registration:** ClinicalTrials.Gov: NCT04291768. Registered on the 24th of February 2020.

## STRENGTHS AND LIMITATIONS

- The trial design - this is a multicenter, randomized, non-inferiority study – which will reduce the risk of confounding bias
- The design of the study strives to be as close to standard clinical practice as possible, which enables the findings of this study to be applicable in a routine clinical setting.
- The strict eligibility criteria will possibly limit the generalizability of the results to all patient groups, as only patients with uncomplicated disease are included.

# INTRODUCTION

## Background

The incidence of Gram-negative bacteremia continues to increase and remains a major cause of morbidity and mortality in both hospitalized and community-dwelling patients.[1] From 1997-2002 the proportion of bacteremia caused by Gram-negative bacteria was 43% in Europe,[2] and a study from the European Antimicrobial Resistance Surveillance System reported an increase in bacteremia due to *Escherichia coli* by 8.1 percent per year from 2002 to 2008.[3] Overall, Gram-negative bacteria account for half of all cases of bacteremia.[4] In Denmark, in 2017 there were > 6000 cases of bacteremia due to the two most common Gram-negative bacteria, *Escherichia coli* and *Klebsiella pneumoniae*.[5]

Prolonged use of antibiotics is closely related to antibiotic-associated infections, antimicrobial resistance, and adverse drug events.[6–9] The latter is particularly concerning for patient safety as it may result in sequelae and prolonged hospital stay. It has been shown that the risk of acute renal failure and the risk of *Clostrioides difficile* infection increase with each day of prophylactic antibiotic treatment prior to surgery,[10] and that for every 10 days of additional antibiotic treatment, the risk of adverse drug events increases by 3%.[9] Antibiotic stewardship and rationale use of antibiotics to treat infections are important strategies that may reduce the duration of antibiotic therapy and thereby reduce adverse events, reduce selective pressure on the bacterial microbiota and prevent the emergence of resistance.[11,12]

The optimal duration of therapy for bloodstream infections due to Gram-negative bacteremia has been poorly defined. International and Danish national recommendations suggest 7-14 days of antibiotic therapy for Gram-negative bacteremia and pyelonephritis according to disease severity.[13–15] However, in the absence of guidelines, wide variability exists, and recommendations are based on individual expert opinions[16] An observational study found that patients receiving short-course (6–10 days) compared to prolonged-course (11–16 days) antibiotic therapy for Gram-negative bacteremia had similar outcomes.[17] Interestingly, there was a trend toward a protective effect of short-course antibiotic therapy on the subsequent emergence of multi-drug resistant Gram-negative bacteremia (odds ratio 0.59; 95% confidence interval 0.32–1.09; P-value 0.09). A recent randomized controlled trial found that an antibiotic course of seven days was non-inferior to 14 days in patients hospitalized with Gram-negative bacteremia achieving clinical stability before day 7.[18] Investigators in Switzerland found that seven days of treatment were non-inferior to 14 days of treatment and that five days of treatment was safe and efficient in the group receiving an individualized duration of treatment determined by clinical response and 75% reduction in peak C-reactive protein values.[19]

Other studies on the duration of treatment in Gram-negative bacteremia also support the safety of shorter antibiotic treatment, but many of these studies have important limitations including small sample sizes, lack of comparator arms, or confounding by indication.[20–24] Randomized controlled trials evaluating the use of procalcitonin in the management of sepsis including those caused by Gram-negative bacteria also demonstrated the safety of shorter antibiotic courses.[20,21]

Given the high stakes of antibiotic overconsumption in an aging population and that only a very few randomized controlled trials have investigated the optimal treatment length of Gram-negative bacteremia, formal evaluation of the safety and efficacy of shortened antibiotic treatment is of immense clinical and public health importance. This study will be designed as a randomized controlled multicenter trial, that will determine whether five days of antibiotic therapy is non-inferior to seven days or longer of therapy for Gram-negative bacteremia. The inclusion of multiple centers, the study design, and inclusion criteria allowing a representative cohort of eligible patients, make it highly likely that the outcomes of this trial will have a significant impact on clinical practice.

### Objective

This study aims to assess the efficacy and safety of shortened antibiotic duration (five days) in the treatment of Gram-negative bacteremia with a urinary tract source of infection in hospitalized immunocompetent adults compared to seven days or more of antibiotic treatment.

## METHODS

### Trial design and randomization

An investigator-initiated multicenter, non-blinded, non-inferiority randomized controlled trial with two parallel treatment arms. One arm will receive shortened antibiotic treatment guided by clinical stability criteria (intervention group) and the other arm will receive standard antibiotic treatment (control group). As the treatment duration relies on continuous evaluation of clinical stability criteria of the participants, blinding of study personnel and participants is not practicable. The design of the study also strives to be as close to standard clinical practice as possible. Therefore, study investigators, trial participants, or treating physicians will not be blinded to treatment allocation.

Confirmation of study eligibility will be performed by entering key variables into a secure web-based program (REDCap) with subsequent automatic patient randomization in the two parallel arms (ratio 1:1) no later than day five after initiation of microbiologically effective empiric antibiotic treatment. The randomization list will be generated centrally in random blocs and stratified according to hospital and etiology. The randomization key will be stored in a locked and secure environment at Copenhagen University Hospital – Hvidovre Hospital.

## Study setting

The following twelve hospitals, representing all five regions of Denmark, will participate in the study: Copenhagen University Hospital – Amager and Hvidovre, Copenhagen University Hospital – Rigshospitalet, Copenhagen University Hospital – Bispebjerg and Frederiksberg, Copenhagen University Hospital – Herlev and Gentofte, Copenhagen University Hospital – North Zealand, Zealand University Hospital – Roskilde, Odense University Hospital, Kolding Hospital, Silkeborg Hospital, Herning Hospital, Aarhus University Hospital, and Aalborg University Hospital.

## Eligibility criteria

Inclusion and exclusion criteria are listed in Table 1.

**Table 1.** *Inclusion and exclusion criteria*

| Inclusion criteria | Exclusion criteria |
|---|---|
| • Age ≥18 years<br>• Blood culture positive for Gram-negative bacteria | • Antibiotic treatment (>2 days) with antimicrobial activity to Gram-negative bacteria within 14 days of inclusion |

| | |
|---|---|
| • Evidence of urinary tract source of infection (positive urine culture with the same bacteria as in the blood culture or at least one clinical symptom compatible with urinary tract infection)<br>• Antibiotic treatment with antimicrobial activity to Gram-negative bacteria administrated within 12 hours of first blood culture<br>• Temperature ≤37.8°C at randomization<br>• Clinically stabile at randomization (systolic blood pressure > 90 mm Hg, heart rate <100 beats/min., respiratory rate <24/minute, peripheral oxygen saturation > 90 %)<br>• Oral and written informed consent | • Gram-negative bacteremia within 30 days of blood culture<br>• Immunosuppression<br>  ○ Untreated HIV-infection<br>  ○ Neutropenia (absolute neutrophil count < 1.0 x $10^9$/l<br>  ○ Untreated terminal cancer<br>  ○ Receiving immunosuppressive agents (ATC-code L04A)<br>  ○ Corticosteroid treatment (≥20 mg/day prednisone or the equivalent for >14 days) within the last 30 days<br>  ○ Chemotherapy within the last 30 days<br>  ○ Immunosuppressed after solid organ transplantation<br>  ○ Asplenia<br>• Polymicrobial growth in blood culture<br>• Bacteremia with non-fermenting Gram-negative bacteria (*Acinetobacter* spp, *Burkholderia* spp, *Pseudomonas* spp), *Brucella* spp, or *Fusobacterium* spp<br>• Failure to remove the source of infection within 72 hours of first blood culture (e.g. change of catheter à demeure)<br>• Pregnancy or breastfeeding |

Gram-negative bacteremia is defined as the growth of a single Gram-negative microorganism in one or more blood cultures associated with evidence of infection. Both community and hospital-acquired Gram-negative bacteremia will be included.

Evidence of a urinary tract source of infection is defined as growth of the same species of Gram-negative microorganism in blood and urine or at least one clinical symptom compatible with urinary tract infection (dysuria, polyuria, hematuria, pelvic pain, cloudy or strong-smelling urine).

Eligible participants must fulfill all the inclusion and none of the exclusion criteria.

## Interventions

Intervention group: will receive antibiotic treatment for five days if clinically stable, i.e. discontinuation of antibiotics at day five if the participant has a temperature of 37.8° C or less and fulfills all criteria of clinical stability at time of randomization. Criteria of clinical stability are systolic blood pressure >90 mm Hg, heart rate <100 beats/min., respiratory rate <24/minute, and peripheral oxygen saturation >90 % without supplemental oxygen.

Control group: will receive antibiotic treatment for a minimum of seven days at the discretion of their treating physician.

## Treatment

Participants will receive antibiotic treatment according to local and national guidelines as well as to antimicrobial susceptibility of the identified Gram-negative bacteria. Participation in the study will

only affect treatment duration and will not influence the choice of treatment concerning the type, dose, or route of administration of antibiotic treatment.

Antibiotics considered appropriate for empiric treatment of Gram-negative bacteremia are listed in Table 2.

**Table 2.** *Acceptable empirical antibiotic treatment of Gram-negative bacteria if susceptible by antibiogram*

| Antibiotic | Administration[1] | Standard dose[1] | Frequency[1] | Dose adjustment[1] |
|---|---|---|---|---|
| *Penicillins* | | | | |
| Piperacillin/Tazobactam | IV | 4 g/0.5 g | Every 6 or 8 hours | Renal impairment and weight |
| Ampicillin[2] | IV | 1-2 g | Every 6 or 8 hours | Renal impairment and weight |
| Mecillinam | IV | 0.8-1 g | Every 8 hours | Renal impairment and weight |
| *Cephalosporins* | | | | |
| Cefuroxime[3] | IV | 1.5 g | Every 6 hours | Renal impairment and weight |
| Cefotaxime[3] | IV | 1 g | Every 12 hours | Renal impairment and weight |
| Ceftazidime | IV | 1 g | Every 8 or 12 hours | Renal impairment and weight |
| Ceftriaxone | IV | 2 g | Every 24 hours | Renal and hepatic impairment and weight |
| *Carbapenems* | | | | |
| Meropenem | IV | 1 g | Every 8 hours | Renal impairment and weight |
| Ertapenem | IV | 1 g | Every 24 hours | - |
| *Aminoglycosides* | | | | |
| Gentamicin[2,3]/ tobramycin | IV | 5-7 mg/kg | Every 24 hours | Renal impairment and weight |

| | | | | |
|---|---|---|---|---|
| *Fluoroquinolone* | | | | |
| Ciprofloxacin | IV | 400 mg | Every 12 hours | Renal impairment and weight |

[1]Standard recommendations, [2]Combination therapy of ampicillin and gentamicin, [3]Monotherapy of cefuroxime/cefotaxime or combination therapy of cefuroxime/cefotaxime and gentamicin

Antibiotics considered appropriate for targeted treatment of Gram-negative bacteremia are listed in Table 3.

**Table 3**. *Acceptable targeted antibiotic treatment of Gram-negative bacteria if susceptible by antibiogram*

| Antibiotic | Administration[1] | Standard dose[1] | Frequency[1] | Dose adjustment[1] |
|---|---|---|---|---|
| *Penicillin* | | | | |
| Mecillinam | IV | 800-1000 mg | Every 8 hours | - |
| Pivmecillinam | PO | 800 mg | Every 8 hours | - |
| Amoxicillin/Clavulanate | PO | 1000 mg/250 mg | Every 6 hours | Renal impairment and weight |
| Pipercillin/Tazobactam | IV | 4 g/0.5 g | Every 6 or 8 hours | Renal impairment and weight |
| Ampicillin | IV | 2 g | Every 6 or 8 hours | Renal impairment and weight |
| Pivampicillin | PO | 500 mg | Every 6 or 8 hours | Renal impairment and weight |
| Amoxicillin | PO | 1 g | Every 6 or 8 hours | Renal impairment and weight |
| *Cephalosporin* | | | | |

| | | | | |
|---|---|---|---|---|
| Cefuroxime | IV | 1.5 g | Every 8 hours | Renal impairment and weight |
| Cefuroxime | PO | 500 mg | Every 12 hours | Renal impairment and weight |
| Cefotaxime | IV | 1 g | Every 8 hours | Renal impairment and weight |
| Ceftazidime | IV | 1 g | Every 8 or 12 hours | Renal impairment and weight |
| Ceftriaxone | IV | 2 g | Every 24 hours | Renal and hepatic impairment and weight |
| *Carbapenem* | | | | |
| Meropenem | IV | 1 g | Every 8 hours | Renal impairment and weight |
| Ertapenem | IV | 1 g | Every 24 hours | - |
| *Aminoglycoside* | | | | |
| Gentamicin/tobramycin | IV | 5-7 mg/kg | Every 24 hours | Renal impairment and weight |
| *Fluoroquinolone* | | | | |
| Ciprofloxacin | IV | 400 mg | Every 12 hours | Renal impairment and weight |
| Ciprofloxacin | PO | 500-750 mg | Every 12 hours | Renal impairment and weight |
| Sulfamethizole | PO | 1 g | Every 12 hours | Renal impairment and weight |
| Nitrofurantoin | PO | 100 mg | Every 6 hours | Renal impairment and weight |
| Trimethoprim | PO | 200 mg | Every 12 hours | Renal impairment and weight |
| Sulfamethoxazole/Trimethoprim | PO | 800 mg/160 mg | Every 12 hours | Renal impairment and weight |

[1]Standard recommendation

Antibiotics will be administered to participants by clinical staff as in usual clinical care during hospitalization. Practical procedures related to antibiotic treatment, including labeling of applied drugs, will follow normal local instructions while the participant is hospitalized. If participants are discharged before the end of therapy, the exact amount of remaining antibiotics will be delivered from the hospital in the original packaging supplied with the additional label. Trained personnel will perform the additional labeling.

Treatment adherence is evaluated by checking medicine administration records of inpatients or by thoroughly interviewing outpatients about their consumption at the planned telephone interview on day 14. Outpatients will be instructed to document self-administrated antibiotic treatment at home to ensure a more accurate measurement of adherence following hospital discharge. Protocol violations will be reported if patients are assessed to be non-compliant (received <80% of scheduled doses).

## Outcomes

Primary outcome

90-day survival without clinical or microbiological failure to treatment as defined:

1. All-cause mortality from the day of randomization until day 90
2. Microbiological failure: Recurrent bacteremia due to the same microorganism as verified by sequence analysis occurring after day 5 of antibiotic treatment and until day 90
3. Clinical failure: Re-initiation of therapy against Gram-negative bacteremia for more than 48 hours due to clinical worsening suspected to be due to the initial infecting organism and for which there is no alternate diagnosis/pathogen suspected from the day of randomization and until day 90

a. Distant complications of initial infection, defined by the growth of the same bacteria as in the initial bacteremia (e.g. endocarditis, meningitis)

b. Local suppurative complication that was not present at infection onset (e.g. renal abscess in pyelonephritis)

Secondary outcomes

- All-cause mortality on days 14, 30 and 90
- Total duration of antibiotic treatment
- Duration and type of antibiotic treatment
- Total length of hospital stay
- Hospital re-admission within 30 and 90 days
- Antibiotic adverse events
- Use of any type of antimicrobials after discharge
- Severe adverse events grade ≥3 as described elsewhere (https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm)
  - Acute kidney injury
- *Clostrioides difficile* infection
- Multidrug-resistance organism

**Participant timeline**

After an initial observation and information period, the participants will be included in the study and randomized no later than day five after the initiation of appropriate empiric antibiotic treatment. Participants will be followed for 90 days. On day 14 (12-16) of follow-up, participants will be

scheduled for blood sample collection and standardized telephone interview. On day 90 (83-97) of follow-up, participants will be scheduled for a final standardized telephone interview. The study flowchart depicts the inclusion, randomization, allocation, follow up and analysis of participants throughout the study, see Figure 1 '*Flow chart on study level'*. The participant timeline is illustrated in Figure 2 '*Flow chart on participant level'*.

## Sample size

Based on a similar previous study, short-term mortality is expected to be approximately 12% in both treatment arms. Failure is expected to be 8% in both study arms[18]. Because individuals who are not stable by day five, who have complicated infections, who have a polymicrobial infection or infection with Pseudomonas spp., Brucella spp., and Fusobacterium spp. are not eligible, we anticipate these rates to be lower at 8% and 4%, respectively. This corresponds to an estimated event rate for the primary outcome of approximately 12%, equivalent to a 90-day survival without clinical or microbiological failure to treatment or relapse of 88 % in both treatment arms.

Non-inferiority is defined as an absolute risk difference or margin in the primary endpoint of up to 10%, as recommended by the European Medicines Agency.[25] Given an $\alpha$ of 5% and a $\beta$ of 90% then 362 randomized individuals are required to be sure that the lower limit of a one-sided 95% confidence interval will exclude a difference in favor of the longer course of antibiotics of more than 10%. Allowing for a dropout rate of 5%, 380 individuals will be included.

A sample size re-estimation (SSR) will be considered at the first planned interim analysis. If the overall event rate falls outside the expected event rate of 12%, an SSR based upon a blinded review of overall data (i.e. without knowledge of the group-specific event rates) will be performed. If the

overall event rate is lower than expected, then the final sample size will be reduced, using the original sample size formula, and replacing the initial estimate with the observed rate. The non-inferiority margin may be reduced to ensure an appropriate margin relative to the event rate. If the overall event rate is higher than expected, the sample size will be increased correspondingly. Any sample size adjustment will be reported to the regulatory authorities as a protocol amendment.

### Recruitment

Investigators and treating physicians at participating sites will identify patients eligible for the trial during days one through four after initiation of empiric antibiotic treatment. Cases are identified by participating Departments of Clinical Microbiology and Infectious Diseases at each center. All participants are hospitalized at enrollment.

The principal investigator will handle questions concerning the recruitment or enrolment of participants, while the study is running.

### Patient and Public Involvement

No patient involved.

## DATA COLLECTION, MANAGEMENT, AND ANALYSIS

### Data collection

At inclusion, data will be obtained from the initial observation period, including baseline diagnostic values, daily vital signs, treatment adherence, and microbiology test results. Furthermore, demographic characteristics will be obtained. Subsequently, participants are scheduled for

standardized telephone consultation on days 12-16 after initiation of empiric antibiotic treatment. A follow-up on days 83-97 will include final standardized telephone consultation, registration of additional antibiotics, readmissions, and vital status. Data collection during the observation and study period is specified in separate sections below and an overview is presented in Table 4.

**Table 4.** *Data collection*

| | OBSERVATION PERIOD[1] | | STUDY PERIOD[2] | |
|---|---|---|---|---|
| | Day 1 | Day 2-5 | Day 14 (12-16) | Day 90 (83-97) |
| Informed consent | | X | | |
| Inclusion | | X | | |
| Randomization | | X | | |
| Demographics[3] | | X | | |
| Comorbidity[4] | | X | | |
| Vital signs[5] | X | X | | |
| Urine culture | X | | | |
| Blood test[6] | X | (X) | X | |
| Microbiology | X | | | X |
| CCI-score[7] | X | | | |
| qSOFA-score[8] | X | X | | |
| PBS[9] | X | X | | |
| Treatment adherence | | X | X | |
| Adverse events | | | X | X |

| | | | | |
|---|---|---|---|---|
| **Additional antibiotics** | | | X | X |
| **Readmission** | | | | X |
| **Vital status** | | | | X |

[1]From day 1 of efficacious empiric antibiotic treatment to inclusion

[2]From inclusion to the end of the trial on day 90

[3]Age, gender, tobacco use, alcohol consumption, medication, medical history, nursing home residency, and activities of daily living

[4]Liver disease, heart disease, congestive heart failure, cerebrovascular disease, renal disease, chronic obstructive pulmonary disorder (COPD), diabetes mellitus, neoplastic disease, hematologic disease, peripheral vascular disease, dementia, connective tissue disease, and ulcer

[5]Blood pressure, heart rate, temperature, respiratory rate, peripheral oxygen saturation

[6]Haemoglobin, leukocytes (WBCs), platelet count, CRP, creatinine, urea, sodium, potassium, bilirubin, alanine aminotransferase, glucose

[7]Charlson's Comorbidity Index: Diabetes with diabetic complications, congestive heart failure, peripheral vascular disease, chronic pulmonary disease, mild and severe liver disease, hemiplegia, renal disease, leukemia, lymphoma, metastatic tumor, and acquired immunodeficiency syndrome (AIDS)[31]

[8]qSOFA score: Glasgow Coma score <15, respiratory rate >22, Systolic BP ≤100

[9]Pitt bacteremia score: Temperature, blood pressure, mental status, respiratory status, cardiac status

## Data management

All data on participants, including demographics, medical history, laboratory and investigational results, will be registered and kept in an electronic case report form (eCRF). The eCRFs will be stored in a secure web application for managing online databases (REDCap (Research Electronic Data Capture)) designed for non-commercial clinical research. There will exist one CRF for each participant for the collection of trial data. Obtained data will be entered manually by investigators or appointed research nurses/assistants into the CRFs. Only personnel associated with the research

project (sponsor, investigators, sub-investigators, and research nurses/assistants) will have encoded access to the CRFs via personal user ID and password.

Sponsor and investigators are obliged to handle all data on trial participants confidentially by the Act on Processing of Personal Data. The primary investigator is responsible for completed CFRs for all trial participants. At the end of the study, the primary investigator will extract data from the electronic database to perform the planned analyses on primary and secondary outcomes. Study data will subsequently be published only in pseudonymous form.

### Statistical methods

Descriptive statistics will be presented as frequency tables, means with standard deviations, or medians with interquartile ranges.

Both intention-to-treat (ITT) and per-protocol (PP) analyses will be performed. Intention-to-treat analysis will comprise all participants including dropouts. Categorical variables will be analyzed with $\chi^2$-test or Fisher's exact test. Continuous variables will be subject to Student's t-test or Wilcoxon rank sum test.

Subgroup-analyses are planned for disease severity, antibiotic group, resistant pathogens, and investigating center. Resistant pathogens are defined as extended-spectrum beta-lactamase (ESBL) producing or carbapenemase-producing Enterobacteriaceae, or pathogens with lack of susceptibility to minimum one agent in three or more classes of antibiotic.

Non-inferiority plots will be performed on the primary outcome for both ITT and PP analyses.

For all statistical analyses except for the non-inferiority analysis, a two-sided p-value <0.05 is considered statistically significant.

The Statistical Analysis Plan is available in Supplemental Material.

## Data monitoring

External monitoring will be performed according to International Conference on Harmonisation-Good Clinical Practice (ICH-GCP). Following a monitoring plan and written standard operating procedures (SOP), monitors will verify that the clinical trial is conducted and generated, documented, and reported in compliance with the protocol, GCP, and applicable regulatory requirements.

The investigating team will provide direct access to all trial-related source data, documents, and reports for monitoring and auditing by the sponsor and inspection by local and regulatory authorities.

The primary endpoint will be evaluated and determined by an independent committee blinded to randomization.

### *Interim analysis*

We will perform interim analyses after the recruitment of every 100 participants. This serves to evaluate primary endpoints and potential adverse events by an independent data and safety monitoring board (DSMB).

The Haybittle-Peto method is applied to demonstrate overwhelming differences between the two

treatment groups that necessitate premature termination of the trial. A significant p-value of 0.001 in the interim analyses will correspond to a p-value of 0.05 in the final analysis.

### Harms

Investigators and sponsor are obliged to follow the study protocol including reporting all adverse events, serious adverse events, and suspected unexpected serious adverse reactions to the relevant authorities as outlined by the Danish Health and Medicine Authority and the European Commission.[26,27]

Participants will be thoroughly asked if they have experienced any adverse event at inclusion and the planned follow-up by phone on days 14 and 90. Adverse events will be registered in predefined CRFs. All adverse events will be followed until they have abated, or until a stable situation has been reached.

All adverse events must be evaluated by investigators and sponsor to determine possible causal association with the antibiotic treatment. At study termination, a final report of registered events in the CRFs will be sent to the Danish Medicines Agency and the Health Research Ethics Committee of the Capital Region of Denmark. Serious adverse reactions, suspected unexpected serious adverse reactions, and information on the general safety of the participants will be listed in an annual safety report to the Danish Medicines Agency and the regional Health Research Ethics Committee.

## DISCUSSION

The results of this study may have major implications on antibiotic use in Gram-negative bacteremia.

Regardless of the results, the study will provide valuable information about current treatment practices by either validating or revising them in light of the scientific evidence. To the best of our knowledge, this is the first study to assess the safety and efficacy of five days of treatment in Gram-negative bacteremia guided by clinical stability criteria. We chose 90-day survival without microbiological and clinical failure as the primary endpoint, as we consider failure and mortality to be the most important measures for clinical safety.

As a potential disadvantage related to shortened antibiotic duration would be the risk of treatment failure, it is therefore crucial to carefully select study participants when considering both efficacy and safety. Accordingly, the inclusion and exclusion criteria of the study were thoroughly based on available data on risk factors related to clinical outcomes. Relying on clinical stability criteria in shortening antibiotic treatment is partly based on a large randomized controlled trial showing that shortened antibiotic treatment against Gram-negative bacteremia is non-inferior to longer antibiotic treatment in patients that reached clinical stability within seven days of treatment.[18] Treatment failure in patients hospitalized with Gram-negative bacteremia has been shown to correlate with initial disease severity, e.g. by Pitt bacteremia score, end-stage liver disease, and immunosuppression.[17] According to protocol, all participants are scheduled for a blood test on day 14 and a standardized telephone interview on day 14, and the last day of follow-up, on day 90, which will ensure early detection of potential treatment failure.

If shortened antibiotic duration in patients hospitalized with Gram-negative bacteremia can be proven to be non-inferior to standard antibiotic treatment, it would likely relieve antibiotic selective pressure and thereby lower the development of bacterial resistance.[3,6,11,12] From the perspective of the

participants, one might expect fewer side effects and better treatment adherence, as prolonged antibiotic use has been associated with an increased risk of side effects.[9] On a community level, this change would lead to a reduction in overall health care costs, as shortened antibiotic duration would result in a decrease in total antibiotic consumption and thereby length of hospital stay.[28–30] As Gram-negative bacteria are accountable for great proportions of bacteremia and thereby antibiotic prescription, this decrease could be quite significant from a national perspective.

## ETHICS AND DISSEMINATION

### Research ethics approval

The study has been approved by Danish National Committee on Health Research (H-19085920), the Danish Medicines Agency (2019-003282-17), and The Danish Data Protection Registry (P-2020-42). The study will be conducted according to ICH-GCP and monitored by GCP units in Denmark.

### Consent or assent

Eligible participants will be scheduled for consultation no later than on day five after initiation of appropriate empiric antibiotic treatment. They will receive both verbal and written information about the study, and subsequently, be offered participation.

Participants will need to sign the informed consent form to be randomized. Participants are asked regardless of initiating empirical treatment at inclusion. The informed consent process for women of childbearing age will include questions on possible pregnancy and will be registered in the eCRF. If there is a possibility of pregnancy, a pregnancy test will be performed with informed consent. As the

intervention period is short (5-14 days) and occurs during hospital admission, it is not anticipated that contraceptive advice is relevant.

The consent form is available in Supplemental Material.

## Post-trial care

All areas of the Danish health care system and all authorized healthcare professionals are covered by a publicly funded compensation scheme. The scheme covers if the participants are injured in connection with treatment at a public hospital. The scheme also covers medicinal product injuries. At inclusion, the participants will be informed by the investigator of the compensation and complaint avenues in case a drug injury arises with the participant, according to *'Lov om klage- og erstatningsadgang inden for sundhedsvæsenet'*.[31]

## Protocol amendments

All protocol amendments have been approved by Danish Regional Committee on Health Research and the Danish Medicines Agency. An overview of protocol changes is shown in Table 5.

**Table 5.** *Summary of protocol changes*

| Protocol version | Protocol changes |
|---|---|
| Version 3,<br>18/02/2020 | Approved for study start |
| Version 4,<br>22/09/2020 | • Exclusion criteria are added:<br>○ Gram-negative bacteremia within the past 30 days.<br>○ Antibiotic treatment >1 day with antimicrobial activity to Gram-negative bacteria within past 14 days. |

| | |
|---|---|
| | ○ Untreated terminal cancer.<br>• Exclusion criteria regarding chemotherapy and immunosuppression are further specified.<br>• Temperature limit for afebrile is changed from 38.0°C to 37.8°C.<br>• Change of site investigator at Copenhagen University Hospital – Gentofte. |
| Version 5,<br>12/11/2021 | • Study period is extended with two years.<br>• Copenhagen University Hospital – Bispebjerg and Frederiksberg is included as study site.<br>• Exclusion criterium regarding antibiotic treatment prior to inclusion is changed from >1 day to >2 days. |

## Access to data

The study is registered at www.clinicaltrials.gov before initiation (ClinicalTrials.Gov: NCT04291768, registered on the 24th of February 2020).

Anonymized trial data will be made available through relevant public databases when the trial ends. On request, anonymized patient-level data, the statistical code, and other relevant supporting information will be made available by contact to the corresponding author.

## Dissemination policy

The data obtained from all participating sites will be pooled and analyzed together as soon as possible after trial completion. Individual researchers will not publish data from the trial until the main study publication has been released. A manuscript with the results of the primary study will be published in a peer-reviewed journal with the primary investigator as the first author, the sponsor as the senior author, and the participating investigators as co-authors according to their work and

involvement in the study.

## Declaration of interests

TB reports grants from Novo Nordisk Foundation, Lundbeck Foundation, Simonsen Foundation, GSK, Pfizer, Gilead, Kai Hansen Foundation and Erik and Susanna Olesen's Charitable Fund; personal fees from GSK, Pfizer, Boehringer Ingelheim, Gilead, MSD, Pentabase ApS, Becton Dickinson, Janssen and Astra Zeneca; outside the submitted work. The remaining authors have no conflicts of interest to declare.

## Author contributions

TB is the sponsor of the study. ST is the coordinating principal investigator. TB conceived the research question of the study. TB and ST obtained the funding for the study. TB, ST, SBI, LTU and CØ participated in the design of the study. ST drafted the study protocol. TB, ST, SBI, LTU, BM, IJ, SL, CØ, PR, KK, and AK contributed to the implementation of the study and revised the protocol critically for important intellectual content. All authors read and approved the final manuscript.

## Acknowledgments

Haakon Sandholt for statistical support.

## Funding Statement

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Research Fund, grant number A6688. Additional federal funding will be sought. Study participants and the Research Ethics Committee of the Capital Region of Denmark will be informed if additional funding has been granted. Sponsor and investigators are independent of economic or competing interests. Participants will not be financially reimbursed. Results from the study are only for scientific and public use and have no commercial interest.

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**Figure 1.** *Flow chart on study level*

**Figure 2.** *Flowchart on participant level*

**Day 1:**
Initiation of microbiologically efficacious empiric antibiotic treatment

**Exclusion:**
- Immunosuppression
- Polymicrobial infection
- Bacteremia with non-fermenting Gram-negative bacteria, *Brucella* spp, or *Fusobacterium* spp
- Failure to remove source of infection
- Pregnancy or breastfeeding

**Inclusion:**
- Age ≥18 years
- Blood culture positive for Gram-negative bacteria
- Evidence of urinary tract source of infection
- Appropriate empiric antibiotic administrated within 12 hours of first blood culture
- Clinically stable at randomization
- T <37.8 at randomization

**Screening period (day 1-4):**
- Demographics
- Baseline values
- Daily monitoring

**Day 3-5:**
Enrollment and randomization

**Allocation 1:1**

**Intervention group:**
5 days of antibiotic treatment

**Control group:**
Antibiotic treatment duration determined by physician, minimum 7 days

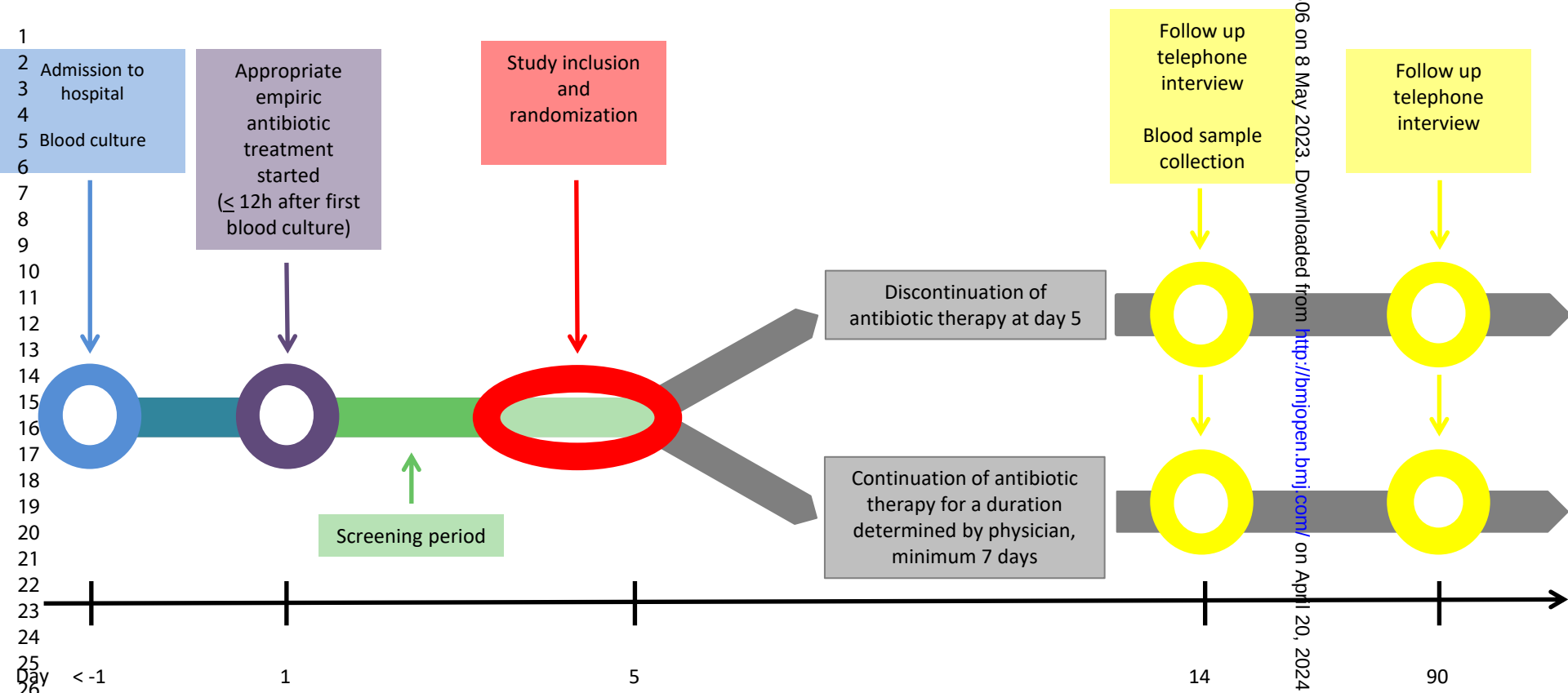
**Follow up**

**Day 14:**
- Structured telephone interview
- Blood sample collection

**Day 14:**
- Structured telephone interview
- Blood sample collection

**Day 90:**
- Structured telephone interview
- Vital status
- Additional antibiotic treatment
- Hospital readmission

**Day 90:**
- Structured telephone interview
- Vital status
- Additional antibiotic treatment
- Hospital readmission



# STATISTICAL ANALYSIS PLAN (SAP)

## Short course antibiotic treatment in Gram-negative bacteremia:

A multicenter, randomized, non-blinded, non-inferiority interventional study

**Eudra-CT no.:** 2019-003282-17

Study protocol version 2, 07-01-2020

SAP version 1, 06-02-2020

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Date and signature

| Statistician | Principal investigator | Sponsor |
|---|---|---|
| 11-2-2020 | 6/2-20 | 6/2-20 |

## 1 INTRODUCTION

This document describes the Statistical Analysis Plan (SAP) for GNB5 trial, a multicenter, randomized, non-blinded clinical trial comparing shortened antibiotic treatment (5 days) with 7 days or longer antibiotic treatment in patients hospitalized with Gram-negative bacteremia with a urinary tract source of infection. It details the statistical method to be used and outlines the planned analyses for the main study.

## 2 ANALYSIS OBJECTIVES

These analyses will assess the efficacy and safety of shortened antibiotic treatment (5 days) in treatment of Gram-negative bacteremia with a urinary tract source of infection in hospitalized immunocompetent adults compared to ≥7 days of antibiotic treatment and will be included in the clinical study report (CSR).

## 3 STUDY METHOD

### 3.1 Trial design

Investigator initiated multicenter, non-blinded, non-inferiority randomized controlled trial with two parallel treatment arms. Randomization will occur in equal proportion (1:1) no later than day 5 of efficacious antibiotic treatment as determined by antibiogram. Participants are stratified by center and etiology.

Intervention group will receive antibiotic treatment for 5 days. The control group will receive antibiotic treatment for a minimum of 7 days at the discretion of their treating physician.

### 3.2 Sample size

We anticipate short-term mortality to be 8% and failure to be 4%. This corresponds to an estimated event rate for the primary outcome of approximately 12%, equivalent to a 90-day survival without clinical or microbiological failure to treatment or relapse of 88 % in both treatment arms.

Non-inferiority is defined as a difference or margin in the primary endpoint of up to 10%. Given an $\alpha$ of 5% and a $\beta$ of 90% then 362 randomized individuals are required to be sure that the lower limit of a one-sided 95% confidence interval will exclude a difference in favor of the longer course of antibiotics of more than 10%. Allowing for a dropout rate of 5%, 380 individuals will be included.

A sample size re-estimation (SSR) will be considered at the first planned interim analysis. If the overall event rate falls outside the expected event rate of 12%, an SSR based upon blinded review of overall

data (i.e. without knowledge of the group-specific event rates) will be performed. If the overall event rate is lower than expected, then the final sample size will be reduced, using the original sample size formula and replacing the initial estimate with the observed rate. The non-inferiority margin may be reduced to ensure an appropriate margin relative to the event rate. If the overall event rate is higher than expected, the sample size will be increased correspondingly. Any sample size adjustment will be reported to the regulatory authorities as a protocol amendment.

### 3.3 Interim analysis

We will perform interim analyses after the recruitment of every 100 participants. This serves to evaluate primary endpoints and potential adverse events by an independent data and safety monitoring board (DSMB).

The Haybittle-Peto method is applied to demonstrate overwhelming differences between the two treatment groups that necessitate premature termination of the trial. A significant p-value of 0.001 in the interim analyses will correspond to a p-value of 0.05 in the final analysis.

### 3.4 Framework

The primary outcome will be tested for non-inferiority. The secondary outcomes will be tested for superiority.

## 4 ANALYSIS SETS

Analyses will be conducted on the following data sets:

### 4.1 Intention-to-treat (ITT) Analysis Data Set

The ITT data set will include all randomized study participants who received at least one dose of study drug regardless of their compliance with the rules of the study. The ITT data set will be used for the analysis of all primary and secondary end points, and all safety-related analysis will be based on the ITT population.

### 4.2 Per protocol (PP) Analysis Data Set

The PP data set will include all randomized participants who received the full duration of study medication according to protocol. Participants with significant variations from the study protocol (e.g. ceasing study drugs early or withdrawal from study for any other reason) will be excluded from the PP

population. Minor procedural variations (e.g. failure to collect additional blood samples at inclusion) will not prelude patients from the PP analysis.

### 4.3 Protocol violations

All protocol violation occurring after randomization will be listed in the Clinical Study Report, tabulated by study ID and investigating center. Dropouts will be included in the ITT population.

## 5 ENDPOINTS

Definitions of study endpoints:

### 5.1 Primary Study Outcome

90-day survival without clinical or microbiological failure to treatment as defined:

1. All-cause mortality from day of randomization and until day 90, with day 1 defined as the date of the initiation of appropriate empiric antibiotic treatment.
2. Microbiological failure: Recurrent bacteremia due to the same microorganism as verified by sequence analysis occurring from after day 5 and until day 90
3. Clinical failure: Re-initiation of therapy against Gram-negative bacteremia for more than 48 hours due to clinical worsening suspected to be due to the initial infecting organism and for which there is no alternate diagnosis/pathogen suspected from the day of randomization and until day 90
    a. Distant complications of initial infection, defined by growth of the same bacteria as in the initial bacteremia (e.g. endocarditis, meningitis)
    b. Local suppurative complication that was not present at infection onset (e.g. renal abscess in pyelonephritis)

### 5.2 Secondary Study Outcomes

To compare shortened antibiotic treatment with longer antibiotic treatment on:

- All-cause mortality at days 14, 30 and 90
- Total duration of antibiotic treatment
- Duration and type of antibiotic treatment
- Total length of hospital stay
- Hospital re-admission within 30 and 90 days
- Antibiotic adverse events
- Use of and type of antimicrobials after discharge

- Severe adverse events grade ≥3
- Acute kidney injury
- *Clostridum difficile* infection
- Multidrug-resistance organism

## 6 STATISTICAL METHODOLOGY

### 6.1 Data validation

Data will be examined for missing values and outliers. Measures of central tendency and dispersion for continuous study parameters will be portrayed. Extreme or unexpected values will be examined individually for authenticity and data discrepancies addressed where appropriate. Additional audit and statistical checks will be performed as necessary.

### 6.2 Missing data

No imputation of missing data will be conducted. Only observed data will be included in the analyses.

### 6.3 Analyses on continuous variables

For continuous variables (e.g. age, duration of antibiotic therapy and hospital stay) results within the treatment arm will be summarized with the number of observations, medians and interquartile ranges or means and standard deviations, depending on distribution. Differences between the control group and interventional group will be calculated using the Wilcoxon Rank-sum test for nonparametric distribution or student's t test for parametric distribution.

### 6.4 Analyses on categorical variables

For categorical variables (e.g. gender, readmissions, mortality) results within the treatment group will be summarized with subject counts and percentages. For endpoints, risk ratios (RR) and the absolute risk difference will be calculated (with 95% confidence intervals). The control group, receiving longer antibiotic treatment, will be used as the reference group. P-values will be based on either Pearson's Chi-square tests or Fischer's exact test. Results may also be represented using forest plots in comparison to the non-inferiority margin, using the Miettinen-Nurminen method.

### 6.5 Endpoint analyses

Both ITT and PP population will be used for both primary and secondary efficacy analyses.

Non-inferiority must be met for the primary analysis of the ITT population for the shortened antibiotic treatment to be regarded as non-inferior to the longer antibiotic treatment. The findings in the PP population must be seen to be consistent in terms of direction and effect size estimates.

The secondary efficacy analyses will be adjusted for multiple testing.

### 6.6 Subgroup analyses

An analysis of the primary and secondary efficacy endpoints is proposed in the following subgroups:

1. Disease severity (given by qSOFA-score and Pitt bacteremia score)
2. Antibiotic group
3. Day of achieved clinical stability (defined as systolic blood pressure $\geq$ 90 mm Hg, heart rate $\leq$100 beats/min., respiratory rate $\leq$24/minute, peripheral oxygen saturation $\geq$ 90 %)
4. Resistant pathogens
5. Investigating center

## 7 STATISTICAL SOFTWARE

Statistical analyses will be performed using R Studio.

# Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

## Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

| | | Reporting Item | Page Number |
|---|---|---|---|
| **Administrative information** | | | |
| Title | #1 | Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym | 1 |

| | | | |
|---|---|---|---|
| Trial registration | #2a | Trial identifier and registry name. If not yet registered, name of intended registry | 1 |
| Trial registration: data set | #2b | All items from the World Health Organization Trial Registration Data Set | N/A |
| Protocol version | #3 | Date and version identifier | 1 |
| Funding | #4 | Sources and types of financial, material, and other support | 13 |
| Roles and responsibilities: contributorship | #5a | Names, affiliations, and roles of protocol contributors | 1+14 |
| Roles and responsibilities: sponsor contact information | #5b | Name and contact information for the trial sponsor | 1+14 |
| Roles and responsibilities: sponsor and funder | #5c | Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities | 14 |
| Roles and responsibilities: committees | #5d | Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and | 14 |

| | | | |
|---|---|---|---|
| | | other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) | |
| **Introduction** | | | |
| Background and rationale | #6a | Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention | 4 |
| Background and rationale: choice of comparators | #6b | Explanation for choice of comparators | 5 |
| Objectives | #7 | Specific objectives or hypotheses | 5 |
| Trial design | #8 | Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory) | 5 |
| **Methods: Participants, interventions, and outcomes** | | | |
| Study setting | #9 | Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained | 6 |

| | | | |
|---|---|---|---|
| Eligibility criteria | #10 | Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) | 6 + Table 1 |
| Interventions: description | #11a | Interventions for each group with sufficient detail to allow replication, including how and when they will be administered | 6 |
| Interventions: modifications | #11b | Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease) | 7 |
| Interventions: adherance | #11c | Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests) | 7 |
| Interventions: concomitant care | #11d | Relevant concomitant care and interventions that are permitted or prohibited during the trial | N/A |
| Outcomes | #12 | Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended | 7 |

| | | | |
|---|---|---|---|
| Participant timeline | #13 | Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) | 8 |
| Sample size | #14 | Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations | 8 |
| Recruitment | #15 | Strategies for achieving adequate participant enrolment to reach target sample size | 9 |
| **Methods: Assignment of interventions (for controlled trials)** | | | |
| Allocation: sequence generation | #16a | Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions | 6 |
| Allocation concealment mechanism | #16b | Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, | N/A |

| | | | |
|---|---|---|---|
| | | sealed envelopes), describing any steps to conceal the sequence until interventions are assigned | |
| Allocation: implementation | #16c | Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions | 6+9 |
| Blinding (masking) | #17a | Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how | 6 |
| Blinding (masking): emergency unblinding | #17b | If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial | N/A |
| **Methods: Data collection, management, and analysis** | | | |
| Data collection plan | #18a | Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol | 9 |

| | | | |
|---|---|---|---|
| Data collection plan: retention | #18b | Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols | N/A |
| Data management | #19 | Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol | 10 |
| Statistics: outcomes | #20a | Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol | 10 |
| Statistics: additional analyses | #20b | Methods for any additional analyses (eg, subgroup and adjusted analyses) | 10 |
| Statistics: analysis population and missing data | #20c | Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) | N/A |
| **Methods: Monitoring** | | | |
| Data monitoring: formal committee | #21a | Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and | 11 |

| | | | |
|---|---|---|---|
| | | competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed | |
| Data monitoring: interim analysis | #21b | Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial | 11 |
| Harms | #22 | Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct | 11 |
| Auditing | #23 | Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor | 11 |
| **Ethics and dissemination** | | | |
| Research ethics approval | #24 | Plans for seeking research ethics committee / institutional review board (REC / IRB) approval | 13 |
| Protocol amendments | #25 | Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators) | 14 |

| | | | |
|---|---|---|---|
| Consent or assent | #26a | Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) | 13 |
| Consent or assent: ancillary studies | #26b | Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable | N/A |
| Confidentiality | #27 | How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial | 10 |
| Declaration of interests | #28 | Financial and other competing interests for principal investigators for the overall trial and each study site | 14 |
| Data access | #29 | Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators | 14 |
| Ancillary and post trial care | #30 | Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation | 13 |
| Dissemination policy: trial results | #31a | Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions | 14 |

| | | | |
|---|---|---|---|
| Dissemination policy: authorship | #31b | Authorship eligibility guidelines and any intended use of professional writers | 14 |
| Dissemination policy: reproducible research | #31c | Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code | 14 |
| **Appendices** | | | |
| Informed consent materials | #32 | Model consent form and other related documentation given to participants and authorised surrogates | Appendix |
| Biological specimens | #33 | Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable | N/A |

BMJ Open

# Short course antibiotic treatment of Gram-negative bacteremia (GNB5): A study protocol for a randomized controlled trial



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| | |

# Short course antibiotic treatment of Gram-negative bacteremia (GNB5): A study protocol for a randomized controlled trial

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## ABSTRACT

**Introduction:** Prolonged use of antibiotics is closely related to antibiotic-associated infections, antimicrobial resistance, and adverse drug events. The optimal duration of antibiotic treatment for Gram-negative bacteremia (GNB) with a urinary tract source of infection is poorly defined.

**Methods and analysis:** Investigator initiated multicenter, non-blinded, non-inferiority randomized controlled trial with two parallel treatment arms. One arm will receive shortened antibiotic treatment of five days and the other arm will receive antibiotic treatment of seven days or longer. Randomization will occur in equal proportion (1:1) no later than day five of effective antibiotic treatment as determined by antibiogram. Immunosuppressed patients and those with GNB due to non-fermenting bacilli (*Acinetobacter* spp., *Pseudomonas* spp.), *Brucella* spp., *Fusobacterium* spp. or polymicrobial growth are ineligible.

The primary endpoint is 90-day survival without clinical or microbiological failure to treatment. Secondary endpoints include all-cause mortality, total duration of antibiotic treatment, hospital

readmission, and *Clostridioides difficile* infection. Interim safety analysis will be performed after the recruitment of every 100 patients. Given an event rate of 12%, a non-inferiority margin of 10%, and 90% power, the required sample size to determine non-inferiority is 380 patients. Analyses will be performed on both intention-to-treat and per-protocol populations.

**Ethics and dissemination:** The study is approved by the Danish Regional Committee on Health Research (H-19085920) and the Danish Medicines Agency (2019-003282-17). The results of the main trial and each of the secondary endpoints will be submitted for publication in a peer-reviewed journal.

**Trial registration:** ClinicalTrials.Gov: NCT04291768. Registered on the 24th of February 2020.

## STRENGTHS AND LIMITATIONS OF THIS STUDY

- The trial design - this is a multicenter, randomized, non-inferiority study – which will reduce the risk of confounding bias
- The design of the study strives to be as close to standard clinical practice as possible, which enables the findings of this study to be applicable in a routine clinical setting.
- The strict eligibility criteria will possibly limit the generalizability of the results to all patient groups, as only patients with uncomplicated disease are included.

# INTRODUCTION

## Background

The incidence of Gram-negative bacteremia continues to increase and remains a major cause of morbidity and mortality in both hospitalized and community-dwelling patients.[1] From 1997-2002 the proportion of bacteremia caused by Gram-negative bacteria was 43% in Europe,[2] and a study from the European Antimicrobial Resistance Surveillance System reported an increase in bacteremia due to *Escherichia coli* by 8.1 percent per year from 2002 to 2008.[3] Overall, Gram-negative bacteria account for half of all cases of bacteremia.[4] In Denmark, in 2017 there were > 6000 cases of bacteremia due to the two most common Gram-negative bacteria, *Escherichia coli* and *Klebsiella pneumoniae*.[5]

Prolonged use of antibiotics is closely related to antibiotic-associated infections, antimicrobial resistance, and adverse drug events.[6–9] The latter is particularly concerning for patient safety as it may result in sequelae and prolonged hospital stay. It has been shown that the risk of acute renal failure and the risk of *Clostridioides difficile* infection increase with each day of prophylactic antibiotic treatment prior to surgery,[10] and that for every 10 days of additional antibiotic treatment, the risk of adverse drug events increases by 3%.[9] Antibiotic stewardship and rationale use of antibiotics to treat infections are important strategies that may reduce the duration of antibiotic therapy and thereby reduce adverse events, reduce selective pressure on the bacterial microbiota and prevent the emergence of resistance.[11,12]

The optimal duration of therapy for bloodstream infections due to Gram-negative bacteremia has been poorly defined. International and Danish national recommendations suggest 7-14 days of antibiotic therapy for Gram-negative bacteremia and pyelonephritis according to disease severity.[13–15] However, in the absence of guidelines, wide variability exists, and recommendations are based on individual expert opinions.[16] An observational study found that patients receiving short-course (6–10 days) compared to prolonged-course (11–16 days) antibiotic therapy for Gram-negative bacteremia had similar outcomes.[17] Interestingly, there was a trend toward a protective effect of short-course antibiotic therapy on the subsequent emergence of multi-drug resistant Gram-negative bacteremia (odds ratio 0.59; 95% confidence interval 0.32–1.09; P-value 0.09). A recent randomized non-inferiority trial found that a seven-day course of antibiotics was the preferential treatment for Gram-negative bacteremia if the source was properly controlled.[18] Another randomized controlled trial found that an antibiotic course of seven days was non-inferior to 14 days in patients hospitalized with Gram-negative bacteremia achieving clinical stability before day seven.[19] Investigators in Switzerland found that seven days of treatment were non-inferior to 14 days of treatment and that five days of treatment was safe and efficient in the group receiving an individualized duration of treatment determined by clinical response and 75% reduction in peak C-reactive protein values.[20]

Other studies on the duration of treatment in Gram-negative bacteremia also support the safety of shorter antibiotic treatment, but many of these studies have important limitations including small sample sizes, lack of comparator arms, or confounding by indication.[21–25] Randomized controlled trials evaluating the use of procalcitonin in the management of sepsis including those caused by Gram-negative bacteria also demonstrated the safety of shorter antibiotic courses.[21,22]

Given the high stakes of antibiotic overconsumption in an aging population and that only a very few randomized controlled trials have investigated the optimal treatment length of Gram-negative bacteremia, formal evaluation of the safety and efficacy of shortened antibiotic treatment is of immense clinical and public health importance. This study will be designed as a randomized controlled multicenter trial, that will determine whether five days of antibiotic therapy is non-inferior to seven days or longer of therapy for Gram-negative bacteremia. The inclusion of multiple centers, the study design, and inclusion criteria allowing a representative cohort of eligible patients, make it highly likely that the outcomes of this trial will have a significant impact on clinical practice.

### Objective

This study aims to assess the efficacy and safety of shortened antibiotic duration (five days) in the treatment of Gram-negative bacteremia with a urinary tract source of infection in hospitalized immunocompetent adults compared to seven days or more of antibiotic treatment.

## METHODS

### Trial design and randomization

An investigator-initiated multicenter, non-blinded, non-inferiority randomized controlled trial with two parallel treatment arms. One arm will receive shortened antibiotic treatment guided by clinical stability criteria (intervention group) and the other arm will receive standard antibiotic treatment (control group). As the treatment duration relies on continuous evaluation of clinical stability criteria of the participants, blinding of study personnel and participants is not practicable. The design of the

study also strives to be as close to standard clinical practice as possible. Therefore, study investigators, trial participants, or treating physicians will not be blinded to treatment allocation.

Confirmation of study eligibility will be performed by entering key variables into a secure web-based program (REDCap) with subsequent automatic patient randomization in the two parallel arms (ratio 1:1) no later than day five after initiation of microbiologically effective empiric antibiotic treatment. The randomization list will be generated centrally in random blocs and stratified according to hospital and etiology. The randomization key will be stored in a locked and secure environment at Copenhagen University Hospital – Hvidovre Hospital.

## Study setting

The following twelve hospitals, representing all five regions of Denmark, will participate in the study: Copenhagen University Hospital – Amager and Hvidovre, Copenhagen University Hospital – Rigshospitalet, Copenhagen University Hospital – Bispebjerg and Frederiksberg, Copenhagen University Hospital – Herlev and Gentofte, Copenhagen University Hospital – North Zealand, Zealand University Hospital – Roskilde, Odense University Hospital, Kolding Hospital, Silkeborg Hospital, Herning Hospital, Aarhus University Hospital, and Aalborg University Hospital.

## Eligibility criteria

Inclusion and exclusion criteria are listed in Table 1.

**Table 1.** *Inclusion and exclusion criteria*

| Inclusion criteria | Exclusion criteria |
|---|---|

| | |
|---|---|
| • Age ≥18 years<br>• Blood culture positive for Gram-negative bacteria<br>• Evidence of urinary tract source of infection (positive urine culture with the same bacteria as in the blood culture or at least one clinical symptom compatible with urinary tract infection)<br>• Antibiotic treatment with antimicrobial activity to Gram-negative bacteria administrated within 12 hours of first blood culture<br>• Temperature ≤37.8°C at randomization<br>• Clinically stabile at randomization (systolic blood pressure > 90 mm Hg, heart rate <100 beats/min., respiratory rate <24/minute, peripheral oxygen saturation > 90 %)<br>• Oral and written informed consent | • Antibiotic treatment (>2 days) with antimicrobial activity to Gram-negative bacteria within 14 days of inclusion<br>• Gram-negative bacteremia within 30 days of blood culture<br>• Immunosuppression<br>  ○ Untreated HIV-infection<br>  ○ Neutropenia (absolute neutrophil count < 1.0 x $10^9$/l<br>  ○ Untreated terminal cancer<br>  ○ Receiving immunosuppressive agents (ATC-code L04A)<br>  ○ Corticosteroid treatment (≥20 mg/day prednisone or the equivalent for >14 days) within the last 30 days<br>  ○ Chemotherapy within the last 30 days<br>  ○ Immunosuppressed after solid organ transplantation<br>  ○ Asplenia<br>• Polymicrobial growth in blood culture<br>• Bacteremia with non-fermenting Gram-negative bacteria (*Acinetobacter* spp, *Burkholderia* spp, *Pseudomonas* spp), *Brucella* spp, or *Fusobacterium* spp |

| | |
|---|---|
| | • Failure to remove the source of infection within 72 hours of first blood culture (e.g. change of indwelling catheter)<br>• Pregnancy or breastfeeding |

Gram-negative bacteremia is defined as the growth of a single Gram-negative microorganism in one or more blood cultures associated with evidence of infection. Both community and hospital-acquired Gram-negative bacteremia will be included.

Evidence of a urinary tract source of infection is defined as growth of the same species of Gram-negative microorganism in blood and urine or at least one clinical symptom compatible with urinary tract infection (dysuria, polyuria, hematuria, pelvic pain, cloudy or strong-smelling urine).

Eligible participants must fulfill all the inclusion and none of the exclusion criteria.

## Interventions

Intervention group: will receive antibiotic treatment for five days if clinically stable, i.e. discontinuation of antibiotics at day five if the participant has a temperature of 37.8° C or less and fulfills all criteria of clinical stability at time of randomization. Criteria of clinical stability are systolic blood pressure >90 mm Hg, heart rate <100 beats/min., respiratory rate <24/minute, and peripheral oxygen saturation >90 % without supplemental oxygen.

Control group: will receive antibiotic treatment for a minimum of seven days at the discretion of their treating physician.

## Treatment

Participants will receive antibiotic treatment according to local and national guidelines as well as to antimicrobial susceptibility of the identified Gram-negative bacteria. Participation in the study will only affect treatment duration and will not influence the choice of treatment concerning the type, dose, or route of administration of antibiotic treatment.

Antibiotics considered appropriate for empiric treatment of Gram-negative bacteremia are listed in Table 2.

**Table 2.** *Acceptable empirical antibiotic treatment of Gram-negative bacteria if susceptible by antibiogram*

| Antibiotic | Administration[1] | Standard dose[1] | Frequency[1] | Dose adjustment[1] |
|---|---|---|---|---|
| *Penicillins* | | | | |
| Piperacillin/Tazobactam | IV | 4 g/0.5 g | Every 6 or 8 hours | Renal impairment and weight |
| Ampicillin[2] | IV | 1-2 g | Every 6 or 8 hours | Renal impairment and weight |
| Mecillinam | IV | 0.8-1 g | Every 8 hours | Renal impairment and weight |
| *Cephalosporins* | | | | |
| Cefuroxime[3] | IV | 1.5 g | Every 6 hours | Renal impairment and weight |
| Cefotaxime[3] | IV | 1 g | Every 12 hours | Renal impairment and weight |
| Ceftazidime | IV | 1 g | Every 8 or 12 | Renal impairment and weight |
| Ceftriaxone | IV | 2 g | hours | Renal and hepatic impairment and |
| | | | Every 24 hours | weight |
| *Carbapenems* | | | | |
| Meropenem | IV | 1 g | Every 8 hours | Renal impairment and weight |

| | | | | |
|---|---|---|---|---|
| Ertapenem | IV | 1 g | Every 24 hours | - |
| *Aminoglycosides* | | | | |
| Gentamicin[2,3]/ tobramycin | IV | 5-7 mg/kg | Every 24 hours | Renal impairment and weight |
| *Fluoroquinolone* | | | | |
| Ciprofloxacin | IV | 400 mg | Every 12 hours | Renal impairment and weight |

[1]Standard recommendations, [2]Combination therapy of ampicillin and gentamicin, [3]Monotherapy of cefuroxime/cefotaxime or combination therapy of cefuroxime/cefotaxime and gentamicin

Antibiotics considered appropriate for targeted treatment of Gram-negative bacteremia are listed in Table 3.

**Table 3**. *Acceptable targeted antibiotic treatment of Gram-negative bacteria if susceptible by antibiogram*

| Antibiotic | Administration[1] | Standard dose[1] | Frequency[1] | Dose adjustment[1] |
|---|---|---|---|---|
| *Penicillin* | | | | |
| Mecillinam | IV | 800-1000 mg | Every 8 hours | - |
| Pivmecillinam | PO | 800 mg | Every 8 hours | - |
| Amoxicillin/Clavulanate | PO | 1000 mg/250 mg | Every 6 hours | Renal impairment and weight |
| Pipercillin/Tazobactam | IV | 4 g/0.5 g | Every 6 or 8 hours | Renal impairment and weight |
| Ampicillin | IV | 2 g | Every 6 or 8 hours | Renal impairment and weight |

| | | | | |
|---|---|---|---|---|
| Pivampicillin | PO | 500 mg | Every 6 or 8 hours | Renal impairment and weight |
| Amoxicillin | PO | 1 g | Every 6 or 8 hours | Renal impairment and weight |
| *Cephalosporin* | | | | |
| Cefuroxime | IV | 1.5 g | Every 8 hours<br>Every 12 hours | Renal impairment and weight |
| Cefuroxime | PO | 500 mg | | Renal impairment and weight |
| Cefotaxime | IV | 1 g | Every 8 hours | Renal impairment and weight |
| Ceftazidime | IV | 1 g | Every 8 or 12 hours | Renal impairment and weight |
| Ceftriaxone | IV | 2 g | Every 24 hours | Renal and hepatic impairment and weight |
| *Carbapenem* | | | | |
| Meropenem | IV | 1 g | Every 8 hours | Renal impairment and weight |
| Ertapenem | IV | 1 g | Every 24 hours | - |
| *Aminoglycoside* | | | | |
| Gentamicin/tobramycin | IV | 5-7 mg/kg | Every 24 hours | Renal impairment and weight |
| *Fluoroquinolone* | | | | |
| Ciprofloxacin | IV | 400 mg | Every 12 hours | Renal impairment and weight |
| Ciprofloxacin | PO | 500-750 mg | Every 12 hours | Renal impairment and weight |
| Sulfamethizole | PO | 1 g | Every 12 hours | Renal impairment and weight |
| Nitrofurantoin | PO | 100 mg | Every 6 hours | Renal impairment and weight |
| Trimethoprim | PO | 200 mg | Every 12 hours | Renal impairment and weight |

| | | | | |
|---|---|---|---|---|
| Sulfamethoxazole/Trimethopr im | PO | 800 mg/160 mg | Every 12 hours | Renal impairment and weight |

[1]Standard recommendation

Antibiotics will be administered to participants by clinical staff as in usual clinical care during hospitalization. Practical procedures related to antibiotic treatment, including labeling of applied drugs, will follow normal local instructions while the participant is hospitalized. If participants are discharged before the end of therapy, the exact amount of remaining antibiotics will be delivered from the hospital in the original packaging supplied with the additional label. Trained personnel will perform the additional labeling.

Treatment adherence is evaluated by checking medicine administration records of inpatients or by thoroughly interviewing outpatients about their consumption at the planned telephone interview on day 14. Outpatients will be instructed to document self-administrated antibiotic treatment at home to ensure a more accurate measurement of adherence following hospital discharge. Protocol violations will be reported if patients are assessed to be non-compliant (received <80% of scheduled doses).

## Outcomes

Primary outcome

90-day survival without clinical or microbiological failure to treatment as defined:

1. All-cause mortality from the day of randomization until day 90
2. Microbiological failure: Recurrent bacteremia due to the same microorganism as verified by sequence analysis occurring after day 5 of antibiotic treatment and until day 90

3. Clinical failure: Re-initiation of therapy against Gram-negative bacteremia for more than 48 hours due to clinical worsening suspected to be due to the initial infecting organism and for which there is no alternate diagnosis/pathogen suspected from the day of randomization and until day 90
    a. Distant complications of initial infection, defined by the growth of the same bacteria as in the initial bacteremia (e.g. endocarditis, meningitis)
    b. Local suppurative complication that was not present at infection onset (e.g. renal abscess in pyelonephritis)

Secondary outcomes

- All-cause mortality on days 14, 30 and 90
- Total duration of antibiotic treatment
- Duration and type of antibiotic treatment
- Total length of hospital stay
- Hospital re-admission within 30 and 90 days
- Antibiotic adverse events
- Use of any type of antimicrobials after discharge
- Severe adverse events grade ≥3 as described elsewhere (https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm)
    - Acute kidney injury
- *Clostridioides s difficile* infection
- Infection with multidrug-resistance organism

A single bacteremic episode was defined as including all positive blood cultures with the same organism within a five-day period, therefor recurrence was defined as occurring after five days of antibiotic treatment and until day 90.

## Participant timeline

After an initial observation and information period, the participants will be included in the study and randomized no later than day five after the initiation of appropriate empiric antibiotic treatment. Participants will be followed for 90 days. On day 14 (12-16) of follow-up, participants will be scheduled for blood sample collection and standardized telephone interview. On day 90 (83-97) of follow-up, participants will be scheduled for a final standardized telephone interview. The study flowchart depicts the inclusion, randomization, allocation, follow up and analysis of participants throughout the study, see Figure 1 '*Flow chart on study level*'. The participant timeline is illustrated in Figure 2 '*Flow chart on participant level*'.

## Sample size

Based on a similar previous study, short-term mortality is expected to be approximately 12% in both treatment arms. Failure is expected to be 8% in both study arms[19]. Because individuals who are not stable by day five, who have complicated infections, who have a polymicrobial infection or infection with Pseudomonas spp., Brucella spp., and Fusobacterium spp. are not eligible, we anticipate these rates to be lower at 8% and 4%, respectively. This corresponds to an estimated event rate for the primary outcome of approximately 12%, equivalent to a 90-day survival without clinical or microbiological failure to treatment or relapse of 88 % in both treatment arms.

Non-inferiority is defined as an absolute risk difference or margin in the primary endpoint of up to 10%, as recommended by the European Medicines Agency.[26] Given an $\alpha$ of 5% and a $\beta$ of 90% then 362 randomized individuals are required to be sure that the lower limit of a one-sided 95% confidence interval will exclude a difference in favor of the longer course of antibiotics of more than 10%. Allowing for a dropout rate of 5%, 380 individuals will be included.

A sample size re-estimation (SSR) will be considered at the first planned interim analysis. If the overall event rate falls outside the expected event rate of 12%, an SSR based upon a blinded review of overall data (i.e. without knowledge of the group-specific event rates) will be performed. If the overall event rate is lower than expected, then the final sample size will be reduced, using the original sample size formula, and replacing the initial estimate with the observed rate. The non-inferiority margin may be reduced to ensure an appropriate margin relative to the event rate. If the overall event rate is higher than expected, the sample size will be increased correspondingly. Any sample size adjustment will be reported to the regulatory authorities as a protocol amendment.

## Recruitment

Investigators and treating physicians at participating sites will identify patients eligible for the trial during days one through four after initiation of empiric antibiotic treatment. Cases are identified by participating Departments of Clinical Microbiology and Infectious Diseases at each center. All participants are hospitalized at enrollment.

The principal investigator will handle questions concerning the recruitment or enrolment of participants, while the study is running.

## Patient and Public Involvement

No patient involved.

## Trial status

The first trial participant was enrolled in March 2020. Recruitment is expected to be completed in February 2026.

## DATA COLLECTION, MANAGEMENT, AND ANALYSIS

### Data collection

At inclusion, data will be obtained from the initial observation period, including baseline diagnostic values, daily vital signs, treatment adherence, and microbiology test results. Furthermore, demographic characteristics will be obtained. Subsequently, participants are scheduled for standardized telephone consultation on days 12-16 after initiation of empiric antibiotic treatment. A follow-up on days 83-97 will include final standardized telephone consultation, registration of additional antibiotics, readmissions, and vital status. Data collection during the observation and study period is specified in separate sections below and an overview is presented in Table 4.

**Table 4.** *Data collection*

| | OBSERVATION PERIOD[1] | | STUDY PERIOD[2] | |
|---|---|---|---|---|
| | Day 1 | Day 2-5 | Day 14 (12-16) | Day 90 (83-97) |

| | | | | |
|---|---|---|---|---|
| **Informed consent** | | X | | |
| **Inclusion** | | X | | |
| **Randomization** | | X | | |
| **Demographics**[3] | | X | | |
| **Comorbidity**[4] | | X | | |
| **Vital signs**[5] | X | X | | |
| **Urine culture** | X | | | |
| **Blood test**[6] | X | (X) | X | |
| **Microbiology** | X | | | X |
| **CCI-score**[7] | X | | | |
| **qSOFA-score**[8] | X | X | | |
| **PBS**[9] | X | X | | |
| **Treatment adherence** | | X | X | |
| **Adverse events** | | | X | X |
| **Additional antibiotics** | | | X | X |
| **Readmission** | | | | X |
| **Vital status** | | | | X |

[1]From day 1 of efficacious empiric antibiotic treatment to inclusion

[2]From inclusion to the end of the trial on day 90

[3]Age, gender, tobacco use, alcohol consumption, medication, medical history, nursing home residency, and activities of daily living

[4]Liver disease, heart disease, congestive heart failure, cerebrovascular disease, renal disease, chronic obstructive pulmonary disorder (COPD), diabetes mellitus, neoplastic disease, hematologic disease, peripheral vascular disease, dementia, connective tissue disease, and ulcer

[5]Blood pressure, heart rate, temperature, respiratory rate, peripheral oxygen saturation

[6]Haemoglobin, leukocytes (WBCs), platelet count, CRP, creatinine, urea, sodium, potassium, bilirubin, alanine aminotransferase, glucose

[7]Charlson's Comorbidity Index: Diabetes with diabetic complications, congestive heart failure, peripheral vascular disease, chronic pulmonary disease, mild and severe liver disease, hemiplegia, renal disease, leukemia, lymphoma, metastatic tumor, and acquired immunodeficiency syndrome (AIDS)[31]

[8]qSOFA score: Glasgow Coma score <15, respiratory rate >22, Systolic BP ≤100

[9]Pitt bacteremia score: Temperature, blood pressure, mental status, respiratory status, cardiac status

## Data management

All data on participants, including demographics, medical history, laboratory and investigational results, will be registered and kept in an electronic case report form (eCRF). The eCRFs will be stored in a secure web application for managing online databases (REDCap (Research Electronic Data Capture)) designed for non-commercial clinical research. There will exist one CRF for each participant for the collection of trial data. Obtained data will be entered manually by investigators or appointed research nurses/assistants into the CRFs. Only personnel associated with the research project (sponsor, investigators, sub-investigators, and research nurses/assistants) will have encoded access to the CRFs via personal user ID and password.

Sponsor and investigators are obliged to handle all data on trial participants confidentially by the Act on Processing of Personal Data. The primary investigator is responsible for completed CFRs for all trial participants. At the end of the study, the primary investigator will extract data from the electronic database to perform the planned analyses on primary and secondary outcomes. Study data will subsequently be published only in pseudonymous form.

## Statistical methods

Descriptive statistics will be presented as frequency tables, means with standard deviations, or medians with interquartile ranges.

Both intention-to-treat (ITT) and per-protocol (PP) analyses will be performed. Intention-to-treat analysis will comprise all participants including dropouts. Categorical variables will be analyzed with $\chi^2$-test or Fisher's exact test. Continuous variables will be subject to Student's t-test or Wilcoxon rank sum test.

Subgroup-analyses are planned for disease severity, antibiotic group, resistant pathogens, and investigating center. Resistant pathogens are defined as extended-spectrum beta-lactamase (ESBL) producing or carbapenemase-producing Enterobacteriaceae, or pathogens with lack of susceptibility to minimum one agent in three or more classes of antibiotic.

Non-inferiority plots will be performed on the primary outcome for both ITT and PP analyses.

For all statistical analyses except for the non-inferiority analysis, a two-sided p-value <0.05 is considered statistically significant.

The Statistical Analysis Plan is available in Supplemental Material.

## Data monitoring

External monitoring will be performed according to International Conference on Harmonisation-Good Clinical Practice (ICH-GCP). Following a monitoring plan and written standard operating procedures

(SOP), monitors will verify that the clinical trial is conducted and generated, documented, and reported in compliance with the protocol, GCP, and applicable regulatory requirements.

The investigating team will provide direct access to all trial-related source data, documents, and reports for monitoring and auditing by the sponsor and inspection by local and regulatory authorities.

The primary endpoint will be evaluated and determined by an independent committee blinded to randomization.

*Interim analysis*

We will perform interim analyses after the recruitment of every 100 participants. This serves to evaluate primary endpoints and potential adverse events by an independent data and safety monitoring board (DSMB).

The Haybittle-Peto method is applied to demonstrate overwhelming differences between the two treatment groups that necessitate premature termination of the trial. A significant p-value of 0.001 in the interim analyses will correspond to a p-value of 0.05 in the final analysis.

## Harms

Investigators and sponsor are obliged to follow the study protocol including reporting all adverse events, serious adverse events, and suspected unexpected serious adverse reactions to the relevant authorities as outlined by the Danish Health and Medicine Authority and the European Commission.[27,28]

Participants will be thoroughly asked if they have experienced any adverse event at inclusion and the planned follow-up by phone on days 14 and 90. Adverse events will be registered in predefined CRFs. All adverse events will be followed until they have abated, or until a stable situation has been reached.

All adverse events must be evaluated by investigators and sponsor to determine possible causal association with the antibiotic treatment. At study termination, a final report of registered events in the CRFs will be sent to the Danish Medicines Agency and the Health Research Ethics Committee of the Capital Region of Denmark. Serious adverse reactions, suspected unexpected serious adverse reactions, and information on the general safety of the participants will be listed in an annual safety report to the Danish Medicines Agency and the regional Health Research Ethics Committee.

## DISCUSSION

The results of this study may have major implications on antibiotic use in Gram-negative bacteremia with urinary tract infection as source of infection. Regardless of the results, the study will provide valuable information about current treatment practices by either validating or revising them in light of the scientific evidence. To the best of our knowledge, this is the first study to assess the safety and efficacy of five days of treatment in Gram-negative bacteremia guided by clinical stability criteria. We chose 90-day survival without microbiological and clinical failure as the primary endpoint, as we consider failure and mortality to be the most important measures for clinical safety.

As a potential disadvantage related to shortened antibiotic duration would be the risk of treatment failure, it is, therefore, crucial to carefully select study participants when considering both efficacy

and safety. Accordingly, the inclusion and exclusion criteria of the study were thoroughly based on available data on risk factors related to clinical outcomes. Relying on clinical stability criteria in shortening antibiotic treatment is partly based on a large randomized controlled trial showing that shortened antibiotic treatment against Gram-negative bacteremia is non-inferior to longer antibiotic treatment in patients that reached clinical stability within seven days of treatment.[19] Treatment failure in patients hospitalized with Gram-negative bacteremia has been shown to correlate with initial disease severity, e.g. by Pitt bacteremia score, end-stage liver disease, and immunosuppression.[17] According to protocol, all participants are scheduled for a blood test on day 14 and a standardized telephone interview on day 14, and the last day of follow-up, on day 90, which will ensure early detection of potential treatment failure.

A limitation to this study is that our strict eligibility criteria will possibly limit the generalizability of the results to all patient groups, as only patients with uncomplicated disease are included.

If shortened antibiotic duration in patients hospitalized with Gram-negative bacteremia can be proven to be non-inferior to standard antibiotic treatment, it would likely relieve antibiotic selective pressure and thereby lower the development of bacterial resistance.[3,6,11,12] From the perspective of the participants, one might expect fewer side effects and better treatment adherence, as prolonged antibiotic use has been associated with an increased risk of side effects.[9] On a community level, this change would lead to a reduction in overall health care costs, as shortened antibiotic duration would result in a decrease in total antibiotic consumption and thereby length of hospital stay.[29–31] As Gram-negative bacteria are accountable for great proportions of bacteremia and thereby antibiotic prescription, this decrease could be quite significant from a national perspective.

## ETHICS AND DISSEMINATION

### Research ethics approval

The study has been approved by Danish National Committee on Health Research (H-19085920), the Danish Medicines Agency (2019-003282-17), and The Danish Data Protection Registry (P-2020-42). The study will be conducted according to ICH-GCP and monitored by GCP units in Denmark.

### Consent or assent

Eligible participants will be scheduled for consultation no later than on day five after initiation of appropriate empiric antibiotic treatment. They will receive both verbal and written information about the study, and subsequently, be offered participation.

Participants will need to sign the informed consent form to be randomized. Participants are asked regardless of initiating empirical treatment at inclusion. The informed consent process for women of childbearing age will include questions on possible pregnancy and will be registered in the eCRF. If there is a possibility of pregnancy, a pregnancy test will be performed with informed consent. As the intervention period is short (5-14 days) and occurs during hospital admission, it is not anticipated that contraceptive advice is relevant.

The consent form is available in Supplemental Material.

### Post-trial care

All areas of the Danish health care system and all authorized healthcare professionals are covered by a publicly funded compensation scheme. The scheme covers if the participants are injured in

connection with treatment at a public hospital. The scheme also covers medicinal product injuries. At inclusion, the participants will be informed by the investigator of the compensation and complaint avenues in case a drug injury arises with the participant, according to *'Lov om klage- og erstatningsadgang inden for sundhedsvæsenet'*.[32]

## Protocol amendments

All protocol amendments have been approved by Danish Regional Committee on Health Research and the Danish Medicines Agency. An overview of protocol changes is shown in Table 5.

**Table 5.** *Summary of protocol changes*

| Protocol version | Protocol changes |
|---|---|
| Version 3,<br>18/02/2020 | Approved for study start |
| Version 4,<br>22/09/2020 | • Exclusion criteria are added:<br>  ○ Gram-negative bacteremia within the past 30 days.<br>  ○ Antibiotic treatment >1 day with antimicrobial activity to Gram-negative bacteria within past 14 days.<br>  ○ Untreated terminal cancer.<br>• Exclusion criteria regarding chemotherapy and immunosuppression are further specified.<br>• Temperature limit for afebrile is changed from 38.0°C to 37.8°C.<br>• Change of site investigator at Copenhagen University Hospital – Gentofte. |
| Version 5,<br>12/11/2021 | • Study period is extended with two years. |

| | |
|---|---|
| | • Copenhagen University Hospital – Bispebjerg and Frederiksberg is included as study site.<br>• Exclusion criterium regarding antibiotic treatment prior to inclusion is changed from >1 day to >2 days. |

## Access to data

The study is registered at www.clinicaltrials.gov before initiation (ClinicalTrials.Gov: NCT04291768, registered on the 24th of February 2020).

Anonymized trial data will be made available through relevant public databases when the trial ends. On request, anonymized patient-level data, the statistical code, and other relevant supporting information will be made available by contact to the corresponding author.

## Dissemination policy

The data obtained from all participating sites will be pooled and analyzed together as soon as possible after trial completion. Individual researchers will not publish data from the trial until the main study publication has been released. A manuscript with the results of the primary study will be published in a peer-reviewed journal with the primary investigator as the first author, the sponsor as the senior author, and the participating investigators as co-authors according to their work and involvement in the study.

## Declaration of interests

TB reports grants from Novo Nordisk Foundation, Lundbeck Foundation, Simonsen Foundation, GSK, Pfizer, Gilead, Kai Hansen Foundation and Erik and Susanna Olesen's Charitable Fund; personal fees from GSK, Pfizer, Boehringer Ingelheim, Gilead, MSD, Pentabase ApS, Becton

Dickinson, Janssen and Astra Zeneca; outside the submitted work. The remaining authors have no conflicts of interest to declare.

## Author contributions

TB is the sponsor of the study. ST is the coordinating principal investigator. TB conceived the research question of the study. TB and ST obtained the funding for the study. TB, ST, SBI, LTU and CØ participated in the design of the study. ST drafted the study protocol. TB, ST, SBI, LTU, BL, IJ, SL, CØ, PR, KK, and AK contributed to the implementation of the study and revised the protocol critically for important intellectual content. All authors read and approved the final manuscript.

## Acknowledgments

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**Figure 1.** *Flow chart on study level*

**Figure 2.** *Flowchart on participant level*

**Day 1:**
Initiation of microbiologically efficacious empiric antibiotic treatment

**Exclusion:**
- Immunosuppression
- Polymicrobial infection
- Bacteremia with non-fermenting Gram-negative bacteria, *Brucella* spp, or *Fusobacterium* spp
- Failure to remove source of infection
- Pregnancy or breastfeeding

**Inclusion:**
- Age ≥18 years
- Blood culture positive for Gram-negative bacteria
- Evidence of urinary tract source of infection
- Appropriate empiric antibiotic administrated within 12 hours of first blood culture
- Clinically stable at randomization
- T <37.8 at randomization

**Screening period (day 1-4):**
- Demographics
- Baseline values
- Daily monitoring

**Day 3-5:**
Enrollment and randomization

**Allocation 1:1**

**Intervention group:**
5 days of antibiotic treatment

**Control group:**
Antibiotic treatment duration determined by physician, minimum 7 days

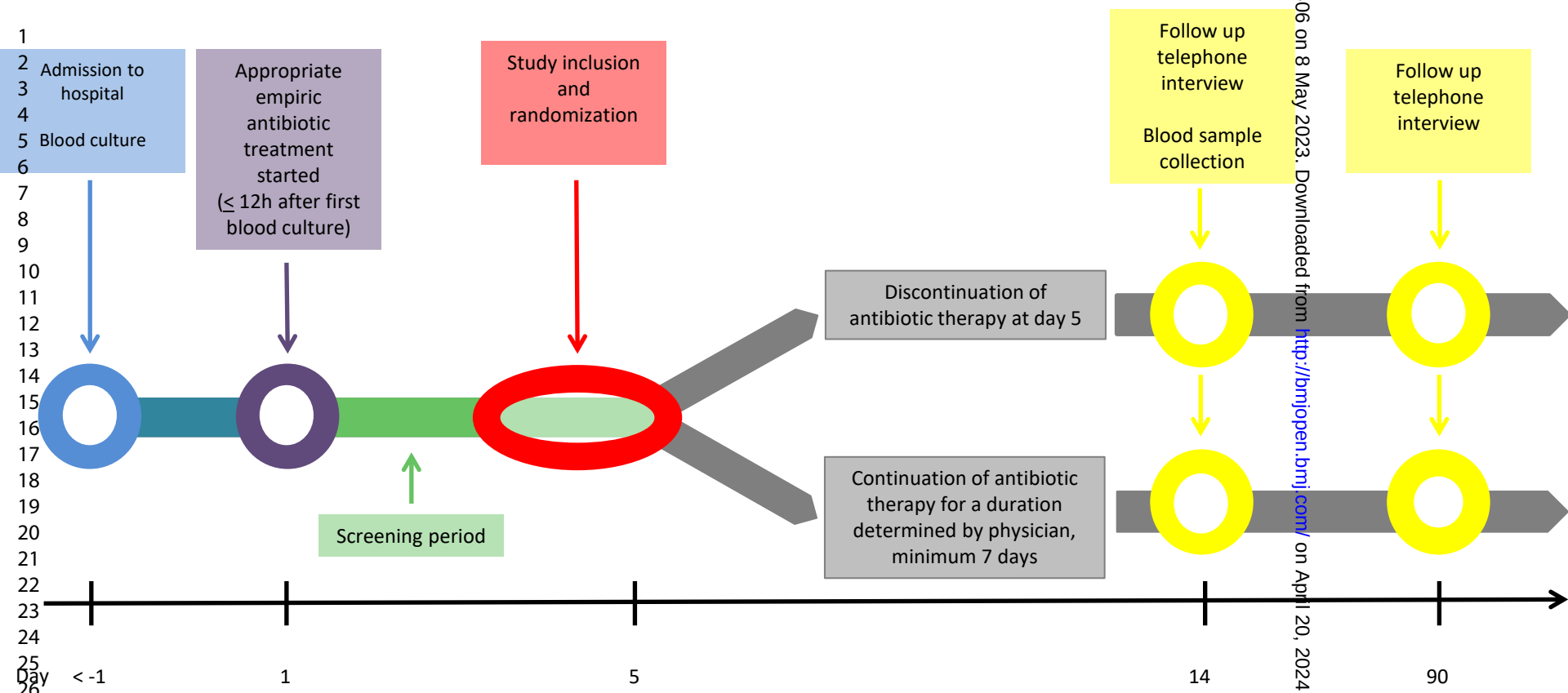
**Follow up**

**Day 14:**
- Structured telephone interview
- Blood sample collection

**Day 14:**
- Structured telephone interview
- Blood sample collection

**Day 90:**
- Structured telephone interview
- Vital status
- Additional antibiotic treatment
- Hospital readmission

**Day 90:**
- Structured telephone interview
- Vital status
- Additional antibiotic treatment
- Hospital readmission



# STATISTICAL ANALYSIS PLAN (SAP)

## Short course antibiotic treatment in Gram-negative bacteremia:

A multicenter, randomized, non-blinded, non-inferiority interventional study

**Eudra-CT no.:** 2019-003282-17

Study protocol version 2, 07-01-2020

SAP version 1, 06-02-2020

**SPONSOR:**

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Date and signature

| | | |
|---|---|---|
| 11-2-2020 | 6/2-20 | 6/2-20 |
| Statistician | Principal investigator | Sponsor |

## 1 INTRODUCTION

This document describes the Statistical Analysis Plan (SAP) for GNB5 trial, a multicenter, randomized, non-blinded clinical trial comparing shortened antibiotic treatment (5 days) with 7 days or longer antibiotic treatment in patients hospitalized with Gram-negative bacteremia with a urinary tract source of infection. It details the statistical method to be used and outlines the planned analyses for the main study.

## 2 ANALYSIS OBJECTIVES

These analyses will assess the efficacy and safety of shortened antibiotic treatment (5 days) in treatment of Gram-negative bacteremia with a urinary tract source of infection in hospitalized immunocompetent adults compared to ≥7 days of antibiotic treatment and will be included in the clinical study report (CSR).

## 3 STUDY METHOD

### 3.1 Trial design

Investigator initiated multicenter, non-blinded, non-inferiority randomized controlled trial with two parallel treatment arms. Randomization will occur in equal proportion (1:1) no later than day 5 of efficacious antibiotic treatment as determined by antibiogram. Participants are stratified by center and etiology.

Intervention group will receive antibiotic treatment for 5 days. The control group will receive antibiotic treatment for a minimum of 7 days at the discretion of their treating physician.

### 3.2 Sample size

We anticipate short-term mortality to be 8% and failure to be 4%. This corresponds to an estimated event rate for the primary outcome of approximately 12%, equivalent to a 90-day survival without clinical or microbiological failure to treatment or relapse of 88 % in both treatment arms.

Non-inferiority is defined as a difference or margin in the primary endpoint of up to 10%. Given an $\alpha$ of 5% and a $\beta$ of 90% then 362 randomized individuals are required to be sure that the lower limit of a one-sided 95% confidence interval will exclude a difference in favor of the longer course of antibiotics of more than 10%. Allowing for a dropout rate of 5%, 380 individuals will be included.

A sample size re-estimation (SSR) will be considered at the first planned interim analysis. If the overall event rate falls outside the expected event rate of 12%, an SSR based upon blinded review of overall

data (i.e. without knowledge of the group-specific event rates) will be performed. If the overall event rate is lower than expected, then the final sample size will be reduced, using the original sample size formula and replacing the initial estimate with the observed rate. The non-inferiority margin may be reduced to ensure an appropriate margin relative to the event rate. If the overall event rate is higher than expected, the sample size will be increased correspondingly. Any sample size adjustment will be reported to the regulatory authorities as a protocol amendment.

### 3.3 Interim analysis

We will perform interim analyses after the recruitment of every 100 participants. This serves to evaluate primary endpoints and potential adverse events by an independent data and safety monitoring board (DSMB).

The Haybittle-Peto method is applied to demonstrate overwhelming differences between the two treatment groups that necessitate premature termination of the trial. A significant p-value of 0.001 in the interim analyses will correspond to a p-value of 0.05 in the final analysis.

### 3.4 Framework

The primary outcome will be tested for non-inferiority. The secondary outcomes will be tested for superiority.

## 4 ANALYSIS SETS

Analyses will be conducted on the following data sets:

### 4.1 Intention-to-treat (ITT) Analysis Data Set

The ITT data set will include all randomized study participants who received at least one dose of study drug regardless of their compliance with the rules of the study. The ITT data set will be used for the analysis of all primary and secondary end points, and all safety-related analysis will be based on the ITT population.

### 4.2 Per protocol (PP) Analysis Data Set

The PP data set will include all randomized participants who received the full duration of study medication according to protocol. Participants with significant variations from the study protocol (e.g. ceasing study drugs early or withdrawal from study for any other reason) will be excluded from the PP

population. Minor procedural variations (e.g. failure to collect additional blood samples at inclusion) will not prelude patients from the PP analysis.

### 4.3 Protocol violations

All protocol violation occurring after randomization will be listed in the Clinical Study Report, tabulated by study ID and investigating center. Dropouts will be included in the ITT population.

## 5 ENDPOINTS

Definitions of study endpoints:

### 5.1 Primary Study Outcome

90-day survival without clinical or microbiological failure to treatment as defined:

1. All-cause mortality from day of randomization and until day 90, with day 1 defined as the date of the initiation of appropriate empiric antibiotic treatment.
2. Microbiological failure: Recurrent bacteremia due to the same microorganism as verified by sequence analysis occurring from after day 5 and until day 90
3. Clinical failure: Re-initiation of therapy against Gram-negative bacteremia for more than 48 hours due to clinical worsening suspected to be due to the initial infecting organism and for which there is no alternate diagnosis/pathogen suspected from the day of randomization and until day 90
    a. Distant complications of initial infection, defined by growth of the same bacteria as in the initial bacteremia (e.g. endocarditis, meningitis)
    b. Local suppurative complication that was not present at infection onset (e.g. renal abscess in pyelonephritis)

### 5.2 Secondary Study Outcomes

To compare shortened antibiotic treatment with longer antibiotic treatment on:

- All-cause mortality at days 14, 30 and 90
- Total duration of antibiotic treatment
- Duration and type of antibiotic treatment
- Total length of hospital stay
- Hospital re-admission within 30 and 90 days
- Antibiotic adverse events
- Use of and type of antimicrobials after discharge

- Severe adverse events grade ≥3
- Acute kidney injury
- *Clostridum difficile* infection
- Multidrug-resistance organism

## 6 STATISTICAL METHODOLOGY

### 6.1 Data validation

Data will be examined for missing values and outliers. Measures of central tendency and dispersion for continuous study parameters will be portrayed. Extreme or unexpected values will be examined individually for authenticity and data discrepancies addressed where appropriate. Additional audit and statistical checks will be performed as necessary.

### 6.2 Missing data

No imputation of missing data will be conducted. Only observed data will be included in the analyses.

### 6.3 Analyses on continuous variables

For continuous variables (e.g. age, duration of antibiotic therapy and hospital stay) results within the treatment arm will be summarized with the number of observations, medians and interquartile ranges or means and standard deviations, depending on distribution. Differences between the control group and interventional group will be calculated using the Wilcoxon Rank-sum test for nonparametric distribution or student's t test for parametric distribution.

### 6.4 Analyses on categorical variables

For categorical variables (e.g. gender, readmissions, mortality) results within the treatment group will be summarized with subject counts and percentages. For endpoints, risk ratios (RR) and the absolute risk difference will be calculated (with 95% confidence intervals). The control group, receiving longer antibiotic treatment, will be used as the reference group. P-values will be based on either Pearson's Chi-square tests or Fischer's exact test. Results may also be represented using forest plots in comparison to the non-inferiority margin, using the Miettinen-Nurminen method.

### 6.5 Endpoint analyses

Both ITT and PP population will be used for both primary and secondary efficacy analyses.

Non-inferiority must be met for the primary analysis of the ITT population for the shortened antibiotic treatment to be regarded as non-inferior to the longer antibiotic treatment. The findings in the PP population must be seen to be consistent in terms of direction and effect size estimates.

The secondary efficacy analyses will be adjusted for multiple testing.

### 6.6 Subgroup analyses

An analysis of the primary and secondary efficacy endpoints is proposed in the following subgroups:

1. Disease severity (given by qSOFA-score and Pitt bacteremia score)
2. Antibiotic group
3. Day of achieved clinical stability (defined as systolic blood pressure $\geq$ 90 mm Hg, heart rate $\leq$100 beats/min., respiratory rate $\leq$24/minute, peripheral oxygen saturation $\geq$ 90 %)
4. Resistant pathogens
5. Investigating center

## 7 STATISTICAL SOFTWARE

Statistical analyses will be performed using R Studio.

# Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

## Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

| | | Reporting Item | Page Number |
|---|---|---|---|
| **Administrative information** | | | |
| Title | #1 | Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym | 1 |

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| Trial registration | #2a | Trial identifier and registry name. If not yet registered, name of intended registry | 1 |
| Trial registration: data set | #2b | All items from the World Health Organization Trial Registration Data Set | N/A |
| Protocol version | #3 | Date and version identifier | 1 |
| Funding | #4 | Sources and types of financial, material, and other support | 13 |
| Roles and responsibilities: contributorship | #5a | Names, affiliations, and roles of protocol contributors | 1+14 |
| Roles and responsibilities: sponsor contact information | #5b | Name and contact information for the trial sponsor | 1+14 |
| Roles and responsibilities: sponsor and funder | #5c | Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities | 14 |
| Roles and responsibilities: committees | #5d | Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and | 14 |

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| | | other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) | |
| **Introduction** | | | |
| Background and rationale | #6a | Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention | 4 |
| Background and rationale: choice of comparators | #6b | Explanation for choice of comparators | 5 |
| Objectives | #7 | Specific objectives or hypotheses | 5 |
| Trial design | #8 | Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory) | 5 |
| **Methods: Participants, interventions, and outcomes** | | | |
| Study setting | #9 | Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained | 6 |

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| Eligibility criteria | #10 | Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) | 6 + Table 1 |
| Interventions: description | #11a | Interventions for each group with sufficient detail to allow replication, including how and when they will be administered | 6 |
| Interventions: modifications | #11b | Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease) | 7 |
| Interventions: adherance | #11c | Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests) | 7 |
| Interventions: concomitant care | #11d | Relevant concomitant care and interventions that are permitted or prohibited during the trial | N/A |
| Outcomes | #12 | Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended | 7 |

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| Participant timeline | #13 | Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) | 8 |
| Sample size | #14 | Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations | 8 |
| Recruitment | #15 | Strategies for achieving adequate participant enrolment to reach target sample size | 9 |
| **Methods: Assignment of interventions (for controlled trials)** | | | |
| Allocation: sequence generation | #16a | Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions | 6 |
| Allocation concealment mechanism | #16b | Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, | N/A |

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| | | sealed envelopes), describing any steps to conceal the sequence until interventions are assigned | |
| Allocation: implementation | #16c | Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions | 6+9 |
| Blinding (masking) | #17a | Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how | 6 |
| Blinding (masking): emergency unblinding | #17b | If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial | N/A |
| **Methods: Data collection, management, and analysis** | | | |
| Data collection plan | #18a | Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol | 9 |

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| Data collection plan: retention | #18b | Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols | N/A |
| Data management | #19 | Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol | 10 |
| Statistics: outcomes | #20a | Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol | 10 |
| Statistics: additional analyses | #20b | Methods for any additional analyses (eg, subgroup and adjusted analyses) | 10 |
| Statistics: analysis population and missing data | #20c | Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) | N/A |
| **Methods: Monitoring** | | | |
| Data monitoring: formal committee | #21a | Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and | 11 |

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| | | competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed | |
| Data monitoring: interim analysis | #21b | Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial | 11 |
| Harms | #22 | Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct | 11 |
| Auditing | #23 | Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor | 11 |
| **Ethics and dissemination** | | | |
| Research ethics approval | #24 | Plans for seeking research ethics committee / institutional review board (REC / IRB) approval | 13 |
| Protocol amendments | #25 | Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators) | 14 |

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| Consent or assent | #26a | Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) | 13 |
| Consent or assent: ancillary studies | #26b | Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable | N/A |
| Confidentiality | #27 | How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial | 10 |
| Declaration of interests | #28 | Financial and other competing interests for principal investigators for the overall trial and each study site | 14 |
| Data access | #29 | Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators | 14 |
| Ancillary and post trial care | #30 | Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation | 13 |
| Dissemination policy: trial results | #31a | Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions | 14 |

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| Dissemination policy: authorship | #31b | Authorship eligibility guidelines and any intended use of professional writers | 14 |
| Dissemination policy: reproducible research | #31c | Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code | 14 |
| **Appendices** | | | |
| Informed consent materials | #32 | Model consent form and other related documentation given to participants and authorised surrogates | Appendix |
| Biological specimens | #33 | Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable | N/A |