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# 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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15 Protocol version: 5.0, 12-NOV-21

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18  
19 Keywords: Randomized controlled trial, Gram-negative bacteremia, bloodstream infection, treatment duration, antibiotic  
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21 stewardship, short-course therapy

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24 Randomized controlled trial, Gram-negative bacteremia, Bloodstream infection, Treatment duration,  
25 Word count: 3816

## 26 27 28 29 **ABSTRACT**

30  
31 **Introduction:** Prolonged use of antibiotics is closely related to antibiotic-associated infections,  
32  
33 antimicrobial resistance, and adverse drug events. The optimal duration of antibiotic treatment for  
34  
35 Gram-negative bacteremia (GNB) with a urinary tract source of infection is poorly defined.

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38 **Methods and analysis:** Investigator initiated multicenter, non-blinded, non-inferiority randomized  
39  
40 controlled trial with two parallel treatment arms. One arm will receive shortened antibiotic treatment  
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42 of five days and the other arm will receive antibiotic treatment of seven days or longer.  
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44 Randomization will occur in equal proportion (1:1) no later than day five of effective antibiotic  
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46 treatment as determined by antibiogram. Immunosuppressed patients and those with GNB due to  
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48 non-fermenting bacilli (*Acinetobacter* spp., *Pseudomonas* spp.), *Brucella* spp., *Fusobacterium* spp.  
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50 or polymicrobial growth are ineligible.  
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55 The primary endpoint is 90-day survival without clinical or microbiological failure to treatment.

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58 Secondary endpoints include all-cause mortality, total duration of antibiotic treatment, hospital  
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4 readmission, and *Clostridioides difficile* infection. Interim safety analysis will be performed after the  
5 recruitment of every 100 patients. Given an event rate of 12%, a non-inferiority margin of 10%, and  
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7 90% power, the required sample size to determine non-inferiority is 380 patients. Analyses will be  
8  
9 performed on both intention-to-treat and per-protocol populations.  
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14 **Ethics and dissemination:** The study is approved by the Danish Regional Committee on Health  
15 Research (H-19085920) and the Danish Medicines Agency (2019-003282-17). The results of the  
16 main trial and each of the secondary endpoints will be submitted for publication in a peer-reviewed  
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18 journal.  
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24 **Trial registration:** ClinicalTrials.Gov: NCT04291768. Registered on the 24<sup>th</sup> of February 2020.  
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## 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.

Peer review only

## 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]

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

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



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4 Given the high stakes of antibiotic overconsumption in an aging population and that only a very few  
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6 randomized controlled trials have investigated the optimal treatment length of Gram-negative  
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8 bacteremia, formal evaluation of the safety and efficacy of shortened antibiotic treatment is of  
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10 immense clinical and public health importance. This study will be designed as a randomized  
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12 controlled multicenter trial, that will determine whether five days of antibiotic therapy is non-inferior  
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14 to seven days or longer of therapy for Gram-negative bacteremia. The inclusion of multiple centers,  
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16 the study design, and inclusion criteria allowing a representative cohort of eligible patients, make it  
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18 highly likely that the outcomes of this trial will have a significant impact on clinical practice.  
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## 25 26 **Objective**

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28 This study aims to assess the efficacy and safety of shortened antibiotic duration (five days) in the  
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30 treatment of Gram-negative bacteremia with a urinary tract source of infection in hospitalized  
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32 immunocompetent adults compared to seven days or more of antibiotic treatment.  
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## 37 38 **METHODS**

### 39 40 **Trial design and randomization**

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42 An investigator-initiated multicenter, non-blinded, non-inferiority randomized controlled trial with two  
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44 parallel treatment arms. One arm will receive shortened antibiotic treatment guided by clinical  
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46 stability criteria (intervention group) and the other arm will receive standard antibiotic treatment  
47  
48 (control group). As the treatment duration relies on continuous evaluation of clinical stability criteria  
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50 of the participants, blinding of study personnel and participants is not practicable. The design of the  
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52 study also strives to be as close to standard clinical practice as possible. Therefore, study  
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54 investigators, trial participants, or treating physicians will not be blinded to treatment allocation.  
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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
<ul style="list-style-type: none"> <li>Age <math>\geq</math>18 years</li> <li>Blood culture positive for Gram-negative bacteria</li> </ul>	<ul style="list-style-type: none"> <li>Antibiotic treatment (&gt;2 days) with antimicrobial activity to Gram-negative bacteria within 14 days of inclusion</li> </ul>

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| <ul style="list-style-type: none"> <li>• Evidence of urinary tract source of infection (positive urine culture with the same bacteria as in the blood culture <u>or</u> at least one clinical symptom compatible with urinary tract infection)</li> <li>• Antibiotic treatment with antimicrobial activity to Gram-negative bacteria administrated within 12 hours of first blood culture</li> <li>• Temperature <math>\leq 37.8^{\circ}\text{C}</math> at randomization</li> <li>• Clinically stable at randomization (systolic blood pressure <math>&gt; 90</math> mm Hg, heart rate <math>&lt; 100</math> beats/min., respiratory rate <math>&lt; 24</math>/minute, peripheral oxygen saturation <math>&gt; 90</math> %)</li> <li>• Oral and written informed consent</li> </ul> | <ul style="list-style-type: none"> <li>• Gram-negative bacteremia within 30 days of blood culture</li> <li>• Immunosuppression <ul style="list-style-type: none"> <li>○ Untreated HIV-infection</li> <li>○ Neutropenia (absolute neutrophil count <math>&lt; 1.0 \times 10^9/l</math>)</li> <li>○ Untreated terminal cancer</li> <li>○ Receiving immunosuppressive agents (ATC-code L04A)</li> <li>○ Corticosteroid treatment (<math>\geq 20</math> mg/day prednisone or the equivalent for <math>&gt; 14</math> days) within the last 30 days</li> <li>○ Chemotherapy within the last 30 days</li> <li>○ Immunosuppressed after solid organ transplantation <ul style="list-style-type: none"> <li>○ Asplenia</li> </ul> </li> </ul> </li> <li>• Polymicrobial growth in blood culture</li> <li>• Bacteremia with non-fermenting Gram-negative bacteria (<i>Acinetobacter</i> spp, <i>Burkholderia</i> spp, <i>Pseudomonas</i> spp), <i>Brucella</i> spp, or <i>Fusobacterium</i> spp)</li> <li>• Failure to remove the source of infection within 72 hours of first blood culture (e.g. change of catheter à demeure)</li> <li>• Pregnancy or breastfeeding</li> </ul> |
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8 Gram-negative bacteremia is defined as the growth of a single Gram-negative microorganism in one  
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10 or more blood cultures associated with evidence of infection. Both community and hospital-acquired  
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12 Gram-negative bacteremia will be included.  
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15 Evidence of a urinary tract source of infection is defined as growth of the same species of Gram-  
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17 negative microorganism in blood and urine or at least one clinical symptom compatible with urinary  
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19 tract infection (dysuria, polyuria, hematuria, pelvic pain, cloudy or strong-smelling urine).  
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23 Eligible participants must fulfill all the inclusion and none of the exclusion criteria.  
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## 28 **Interventions**

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30 Intervention group: will receive antibiotic treatment for five days if clinically stable, i.e. discontinuation  
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32 of antibiotics at day five if the participant has a temperature of 37.8° C or less and fulfills all criteria  
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34 of clinical stability at time of randomization. Criteria of clinical stability are systolic blood pressure  
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36 >90 mm Hg, heart rate <100 beats/min., respiratory rate <24/minute, and peripheral oxygen  
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38 saturation >90 % without supplemental oxygen.  
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43 Control group: will receive antibiotic treatment for a minimum of seven days at the discretion of their  
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45 treating physician.  
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## 50 **Treatment**

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52 Participants will receive antibiotic treatment according to local and national guidelines as well as to  
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54 antimicrobial susceptibility of the identified Gram-negative bacteria. Participation in the study will  
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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 <sup>n1</sup>	Standard dose <sup>1</sup>	Frequency <sup>1</sup>	Dose adjustment <sup>1</sup>
<i>Penicillins</i>				
Piperacillin/Tazobactam	IV	4 g/0.5 g	Every 6 or 8 hours	Renal impairment and weight
Ampicillin <sup>2</sup>	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
<i>Cephalosporins</i>				
Cefuroxime <sup>3</sup>	IV	1.5 g	Every 6 hours	Renal impairment and weight
Cefotaxime <sup>3</sup>	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
<i>Carbapenems</i>				
Meropenem	IV	1 g	Every 8 hours	Renal impairment and weight
Ertapenem	IV	1 g	Every 24 hours	-
<i>Aminoglycosides</i>				
Gentamicin <sup>2,3/</sup>	IV	5-7 mg/kg	Every 24 hours	Renal impairment and weight
tobramycin				

*Fluoroquinolone*

Ciprofloxacin	IV	400 mg	Every 12 hours	Renal impairment and weight
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<sup>1</sup>Standard recommendations, <sup>2</sup>Combination therapy of ampicillin and gentamicin, <sup>3</sup>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 <sup>1</sup>	Standard dose <sup>1</sup>	Frequency <sup>1</sup>	Dose adjustment <sup>1</sup>
<i>Penicillin</i>				
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
<i>Cephalosporin</i>				

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				Every 8 hours	
	Cefuroxime	IV	1.5 g		Renal impairment and weight
				Every 12 hours	
	Cefuroxime	PO	500 mg		Renal impairment and weight
				Every 8 hours	
	Cefotaxime	IV	1 g		Renal impairment and weight
				Every 8 or 12	
	Ceftazidime	IV	1 g		Renal impairment and weight
				hours	
					Renal and hepatic impairment
	Ceftriaxone	IV	2 g	Every 24 hours	and weight
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	<i>Carbapenem</i>				
	Meropenem	IV	1 g	Every 8 hours	Renal impairment and weight
	Ertapenem	IV	1 g	Every 24 hours	-
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	<i>Aminoglycoside</i>				
	Gentamicin/tobramycin	IV	5-7 mg/kg	Every 24 hours	Renal impairment and weight
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	<i>Fluoroquinolone</i>				
	Ciprofloxacin	IV	400 mg	Every 12 hours	Renal impairment and weight
	Ciprofloxacin	PO	500-750 mg	Every 12 hours	Renal impairment and weight
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	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				
		PO	800 mg/160 mg	Every 12 hours	Renal impairment and weight
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<sup>1</sup>Standard recommendation

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4 Antibiotics will be administered to participants by clinical staff as in usual clinical care during  
5 hospitalization. Practical procedures related to antibiotic treatment, including labeling of applied  
6 drugs, will follow normal local instructions while the participant is hospitalized. If participants are  
7 discharged before the end of therapy, the exact amount of remaining antibiotics will be delivered  
8 from the hospital in the original packaging supplied with the additional label. Trained personnel will  
9 perform the additional labeling.  
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21 Treatment adherence is evaluated by checking medicine administration records of inpatients or by  
22 thoroughly interviewing outpatients about their consumption at the planned telephone interview on  
23 day 14. Outpatients will be instructed to document self-administrated antibiotic treatment at home to  
24 ensure a more accurate measurement of adherence following hospital discharge. Protocol violations  
25 will be reported if patients are assessed to be non-compliant (received <80% of scheduled doses).  
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## 36 Outcomes

### 37 Primary outcome

38 90-day survival without clinical or microbiological failure to treatment as defined:

- 39 1. All-cause mortality from the day of randomization until day 90
  - 40 2. Microbiological failure: Recurrent bacteremia due to the same microorganism as verified by  
41 sequence analysis occurring after day 5 of antibiotic treatment and until day 90
  - 42 3. Clinical failure: Re-initiation of therapy against Gram-negative bacteremia for more than 48  
43 hours due to clinical worsening suspected to be due to the initial infecting organism and for  
44 which there is no alternate diagnosis/pathogen suspected from the day of randomization and  
45 until day 90
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- 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  $\geq 3$  as described elsewhere ([https://ctep.cancer.gov/protocoldevelopment/electronic\\_applications/ctc.htm](https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm))
- Acute kidney injury
- *Clostridioides 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

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

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21 Based on a similar previous study, short-term mortality is expected to be approximately 12% in both  
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23 treatment arms. Failure is expected to be 8% in both study arms[18]. Because individuals who are  
24  
25 not stable by day five, who have complicated infections, who have a polymicrobial infection or  
26  
27 infection with *Pseudomonas* spp., *Brucella* spp., and *Fusobacterium* spp. are not eligible, we  
28  
29 anticipate these rates to be lower at 8% and 4%, respectively. This corresponds to an estimated  
30  
31 event rate for the primary outcome of approximately 12%, equivalent to a 90-day survival without  
32  
33 clinical or microbiological failure to treatment or relapse of 88 % in both treatment arms.  
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38 Non-inferiority is defined as an absolute risk difference or margin in the primary endpoint of up to  
39  
40 10%, as recommended by the European Medicines Agency.[25] Given an  $\alpha$  of 5% and a  $\beta$  of 90%  
41  
42 then 362 randomized individuals are required to be sure that the lower limit of a one-sided 95%  
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44 confidence interval will exclude a difference in favor of the longer course of antibiotics of more than  
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46 10%. Allowing for a dropout rate of 5%, 380 individuals will be included.  
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52 A sample size re-estimation (SSR) will be considered at the first planned interim analysis. If the  
53  
54 overall event rate falls outside the expected event rate of 12%, an SSR based upon a blinded review  
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56 of overall data (i.e. without knowledge of the group-specific event rates) will be performed. If the  
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4 overall event rate is lower than expected, then the final sample size will be reduced, using the original  
5 sample size formula, and replacing the initial estimate with the observed rate. The non-inferiority  
6 margin may be reduced to ensure an appropriate margin relative to the event rate. If the overall event  
7 rate is higher than expected, the sample size will be increased correspondingly. Any sample size  
8 adjustment will be reported to the regulatory authorities as a protocol amendment.  
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### 19 **Recruitment**

20  
21 Investigators and treating physicians at participating sites will identify patients eligible for the trial  
22 during days one through four after initiation of empiric antibiotic treatment. Cases are identified by  
23 participating Departments of Clinical Microbiology and Infectious Diseases at each center. All  
24 participants are hospitalized at enrollment.  
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31 The principal investigator will handle questions concerning the recruitment or enrolment of  
32 participants, while the study is running.  
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### 40 **Patient and Public Involvement**

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43 No patient involved.  
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## 48 **DATA COLLECTION, MANAGEMENT, AND ANALYSIS**

### 49 **Data collection**

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52 At inclusion, data will be obtained from the initial observation period, including baseline diagnostic  
53 values, daily vital signs, treatment adherence, and microbiology test results. Furthermore,  
54 demographic characteristics will be obtained. Subsequently, participants are scheduled for  
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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 <sup>1</sup>		STUDY PERIOD <sup>2</sup>	
	Day 1	Day 2-5	Day 14 (12-16)	Day 90 (83-97)
Informed consent		X		
Inclusion		X		
Randomization		X		
Demographics <sup>3</sup>		X		
Comorbidity <sup>4</sup>		X		
Vital signs <sup>5</sup>	X	X		
Urine culture	X			
Blood test <sup>6</sup>	X	(X)	X	
Microbiology	X			X
CCI-score <sup>7</sup>	X			
qSOFA-score <sup>8</sup>	X	X		
PBS <sup>9</sup>	X	X		
Treatment adherence		X	X	
Adverse events			X	X

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<b>Additional antibiotics</b>			X	X
<b>Readmission</b>				X
<b>Vital status</b>				X

<sup>1</sup>From day 1 of efficacious empiric antibiotic treatment to inclusion

<sup>2</sup>From inclusion to the end of the trial on day 90

<sup>3</sup>Age, gender, tobacco use, alcohol consumption, medication, medical history, nursing home residency, and activities of daily living

<sup>4</sup>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

<sup>5</sup>Blood pressure, heart rate, temperature, respiratory rate, peripheral oxygen saturation

<sup>6</sup>Haemoglobin, leukocytes (WBCs), platelet count, CRP, creatinine, urea, sodium, potassium, bilirubin, alanine aminotransferase, glucose

<sup>7</sup>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)<sup>31</sup>

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

<sup>9</sup>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

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4 project (sponsor, investigators, sub-investigators, and research nurses/assistants) will have encoded  
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6 access to the CRFs via personal user ID and password.  
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11 Sponsor and investigators are obliged to handle all data on trial participants confidentially by the Act  
12 on Processing of Personal Data. The primary investigator is responsible for completed CFRs for all  
13 trial participants. At the end of the study, the primary investigator will extract data from the electronic  
14 database to perform the planned analyses on primary and secondary outcomes. Study data will  
15 subsequently be published only in pseudonymous form.  
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## 26 **Statistical methods**

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28 Descriptive statistics will be presented as frequency tables, means with standard deviations, or  
29 medians with interquartile ranges.  
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35 Both intention-to-treat (ITT) and per-protocol (PP) analyses will be performed. Intention-to-treat  
36 analysis will comprise all participants including dropouts. Categorical variables will be analyzed with  
37  $\chi^2$ -test or Fisher's exact test. Continuous variables will be subject to Student's t-test or Wilcoxon rank  
38 sum test.  
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48 Subgroup-analyses are planned for disease severity, antibiotic group, resistant pathogens, and  
49 investigating center. Resistant pathogens are defined as extended-spectrum beta-lactamase (ESBL)  
50 producing or carbapenemase-producing Enterobacteriaceae, or pathogens with lack of susceptibility  
51 to minimum one agent in three or more classes of antibiotic.  
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4 Non-inferiority plots will be performed on the primary outcome for both ITT and PP analyses.  
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9 For all statistical analyses except for the non-inferiority analysis, a two-sided p-value  $<0.05$  is  
10 considered statistically significant.  
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16 The Statistical Analysis Plan is available in Supplemental Material.  
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### 21 **Data monitoring**

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23 External monitoring will be performed according to International Conference on Harmonisation-Good  
24 Clinical Practice (ICH-GCP). Following a monitoring plan and written standard operating procedures  
25 (SOP), monitors will verify that the clinical trial is conducted and generated, documented, and  
26 reported in compliance with the protocol, GCP, and applicable regulatory requirements.  
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33 The investigating team will provide direct access to all trial-related source data, documents, and  
34 reports for monitoring and auditing by the sponsor and inspection by local and regulatory authorities.  
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40 The primary endpoint will be evaluated and determined by an independent committee blinded to  
41 randomization.  
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### 48 *Interim analysis*

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50 We will perform interim analyses after the recruitment of every 100 participants. This serves to  
51 evaluate primary endpoints and potential adverse events by an independent data and safety  
52 monitoring board (DSMB).  
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58 The Haybittle-Peto method is applied to demonstrate overwhelming differences between the two  
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4 treatment groups that necessitate premature termination of the trial. A significant p-value of 0.001 in  
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7 the interim analyses will correspond to a p-value of 0.05 in the final analysis.  
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## 10 11 **Harms**

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14 Investigators and sponsor are obliged to follow the study protocol including reporting all adverse  
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16 events, serious adverse events, and suspected unexpected serious adverse reactions to the relevant  
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18 authorities as outlined by the Danish Health and Medicine Authority and the European  
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20 Commission.[26,27]  
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26 Participants will be thoroughly asked if they have experienced any adverse event at inclusion and  
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28 the planned follow-up by phone on days 14 and 90. Adverse events will be registered in predefined  
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30 CRFs. All adverse events will be followed until they have abated, or until a stable situation has been  
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32 reached.  
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38 All adverse events must be evaluated by investigators and sponsor to determine possible causal  
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40 association with the antibiotic treatment. At study termination, a final report of registered events in  
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42 the CRFs will be sent to the Danish Medicines Agency and the Health Research Ethics Committee  
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44 of the Capital Region of Denmark. Serious adverse reactions, suspected unexpected serious  
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46 adverse reactions, and information on the general safety of the participants will be listed in an annual  
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48 safety report to the Danish Medicines Agency and the regional Health Research Ethics Committee.  
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## 52 53 54 **DISCUSSION**

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

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

### 24 **Research ethics approval**

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

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40 Eligible participants will be scheduled for consultation no later than on day five after initiation of  
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42 appropriate empiric antibiotic treatment. They will receive both verbal and written information about  
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44 the study, and subsequently, be offered participation.  
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48 Participants will need to sign the informed consent form to be randomized. Participants are asked  
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50 regardless of initiating empirical treatment at inclusion. The informed consent process for women of  
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52 childbearing age will include questions on possible pregnancy and will be registered in the eCRF. If  
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54 there is a possibility of pregnancy, a pregnancy test will be performed with informed consent. As the  
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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*.<sup>[31]</sup>

### 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, 18/02/2020	Approved for study start
Version 4, 22/09/2020	<ul style="list-style-type: none"> <li>• Exclusion criteria are added:               <ul style="list-style-type: none"> <li>○ Gram-negative bacteremia within the past 30 days.</li> <li>○ Antibiotic treatment &gt;1 day with antimicrobial activity to Gram-negative bacteria within past 14 days.</li> </ul> </li> </ul>

	<ul style="list-style-type: none"> <li>○ Untreated terminal cancer.</li> <li>• Exclusion criteria regarding chemotherapy and immunosuppression are further specified.</li> <li>• Temperature limit for afebrile is changed from 38.0°C to 37.8°C.</li> <li>• Change of site investigator at Copenhagen University Hospital – Gentofte.</li> </ul>
Version 5, 12/11/2021	<ul style="list-style-type: none"> <li>• Study period is extended with two years.</li> <li>• Copenhagen University Hospital – Bispebjerg and Frederiksberg is included as study site.</li> <li>• Exclusion criterium regarding antibiotic treatment prior to inclusion is changed from &gt;1 day to &gt;2 days.</li> </ul>

### Access to data

The study is registered at [www.clinicaltrials.gov](http://www.clinicaltrials.gov) before initiation (ClinicalTrials.Gov: NCT04291768, registered on the 24<sup>th</sup> 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

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4 involvement in the study.  
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### 9 **Declaration of interests**

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11 TB reports grants from Novo Nordisk Foundation, Lundbeck Foundation, Simonsen Foundation,  
12  
13 GSK, Pfizer, Gilead, Kai Hansen Foundation and Erik and Susanna Olesen's Charitable Fund;  
14  
15 personal fees from GSK, Pfizer, Boehringer Ingelheim, Gilead, MSD, Pentabase ApS, Becton  
16  
17 Dickinson, Janssen and Astra Zeneca; outside the submitted work. The remaining authors have no  
18  
19 conflicts of interest to declare.  
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### 27 **Author contributions**

28  
29 TB is the sponsor of the study. ST is the coordinating principal investigator. TB conceived the  
30  
31 research question of the study. TB and ST obtained the funding for the study. TB, ST, SBI, LTU and  
32  
33 CØ participated in the design of the study. ST drafted the study protocol. TB, ST, SBI, LTU, BM, IJ,  
34  
35 SL, CØ, PR, KK, and AK contributed to the implementation of the study and revised the protocol  
36  
37 critically for important intellectual content. All authors read and approved the final manuscript.  
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### 45 **Acknowledgments**

46  
47 Haakon Sandholt for statistical support.  
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53  
54 The study is partly financially supported by a grant from Hvidovre Hospital Strategic Research Fund,  
55  
56 Danish Regions Medicine Grant, grant number EMN-2019-01055, and Capital Region of Denmark  
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4 Research Fund, grant number A6688. Additional federal funding will be sought. Study participants  
5  
6 and the Research Ethics Committee of the Capital Region of Denmark will be informed if additional  
7  
8 funding has been granted. Sponsor and investigators are independent of economic or competing  
9  
10 interests. Participants will not be financially reimbursed. Results from the study are only for scientific  
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12 and public use and have no commercial interest.  
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For peer review only

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

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

For peer review only

**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  $\geq 18$  years
- Blood culture positive for Gram-negative bacteria
- Evidence of urinary tract source of infection
- Appropriate empiric antibiotic administered 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

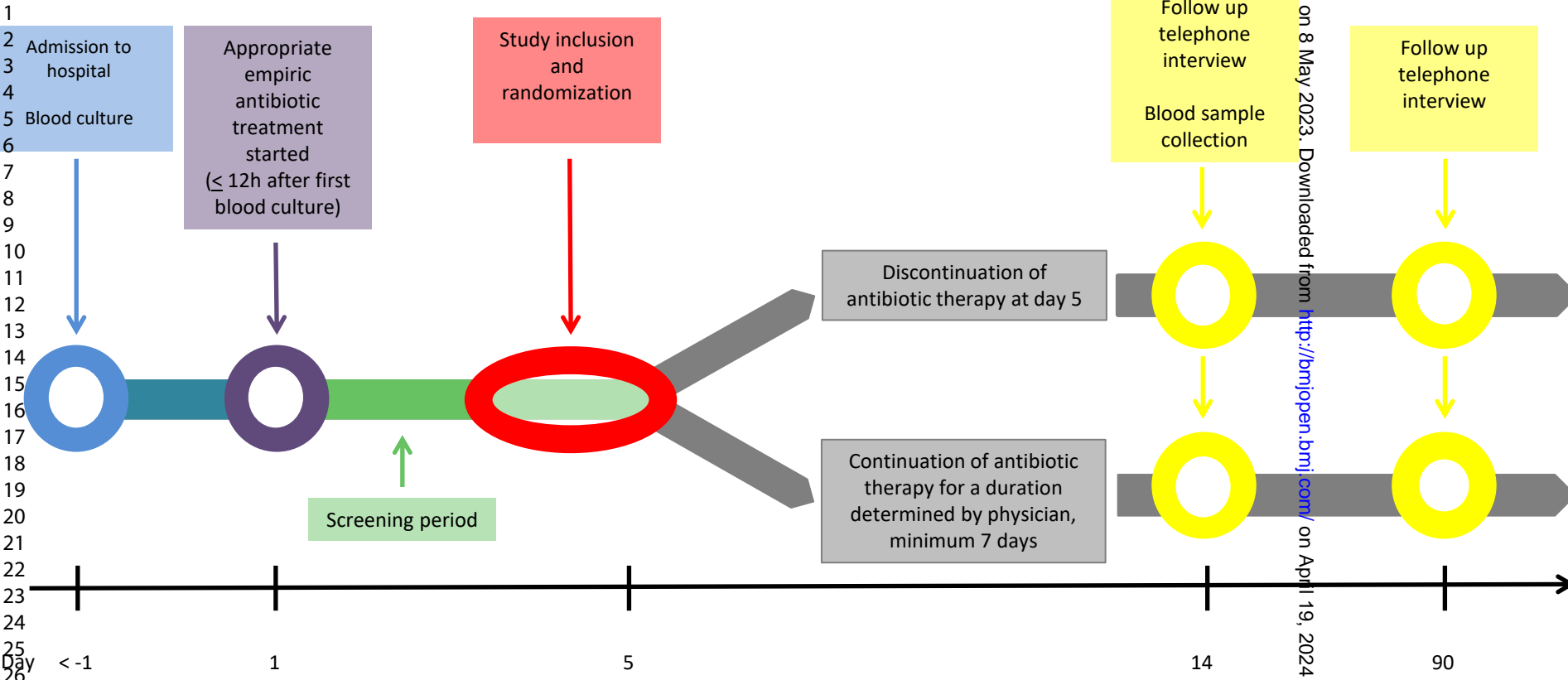
**Follow up**

**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



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Eudra-CT no.: 2019-003282-17

GNB5

# 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

11-2-2020  


Statistician

6/2-20  


Principal investigator

6/2-20  


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  $\geq 7$  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

Eudra-CT no.: 2019-003282-17

GNBS

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 preclude 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  $\geq 3$
- Acute kidney injury
- *Clostridium 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.

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3 Non-inferiority must be met for the primary analysis of the ITT population for the shortened antibiotic  
4 treatment to be regarded as non-inferior to the longer antibiotic treatment. The findings in the PP  
5 population must be seen to be consistent in terms of direction and effect size estimates.  
6

7 The secondary efficacy analyses will be adjusted for multiple testing.  
8  
9

## 10 11 **6.6 Subgroup analyses**

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

- 13 1. Disease severity (given by qSOFA-score and Pitt bacteremia score)
  - 14 2. Antibiotic group
  - 15 3. Day of achieved clinical stability (defined as systolic blood pressure  $\geq$  90 mm Hg, heart rate  
16  $\leq$ 100 beats/min., respiratory rate  $\leq$ 24/minute, peripheral oxygen saturation  $\geq$  90 %)
  - 17 4. Resistant pathogens
  - 18 5. Investigating center
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## 26 **7 STATISTICAL SOFTWARE**

27 Statistical analyses will be performed using R Studio.  
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# 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 SPIRIT reporting 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

		Page
	Reporting Item	Number
<b>Administrative information</b>		
Title	<a href="#">#1</a> Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1

1	Trial registration	<a href="#">#2a</a>	Trial identifier and registry name. If not yet registered,	1
2			name of intended registry	
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6	Trial registration:	<a href="#">#2b</a>	All items from the World Health Organization Trial	N/A
7				
8	data set		Registration Data Set	
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11				
12	Protocol version	<a href="#">#3</a>	Date and version identifier	1
13				
14				
15	Funding	<a href="#">#4</a>	Sources and types of financial, material, and other	13
16			support	
17				
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19				
20	Roles and	<a href="#">#5a</a>	Names, affiliations, and roles of protocol contributors	1+14
21				
22	responsibilities:			
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24	contributorship			
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28	Roles and	<a href="#">#5b</a>	Name and contact information for the trial sponsor	1+14
29				
30	responsibilities:			
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32	sponsor contact			
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34	information			
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38	Roles and	<a href="#">#5c</a>	Role of study sponsor and funders, if any, in study	14
39			design; collection, management, analysis, and	
40	responsibilities:		interpretation of data; writing of the report; and the	
41			decision to submit the report for publication, including	
42	sponsor and funder		whether they will have ultimate authority over any of	
43			these activities	
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52	Roles and	<a href="#">#5d</a>	Composition, roles, and responsibilities of the	14
53			coordinating centre, steering committee, endpoint	
54	responsibilities:		adjudication committee, data management team, and	
55				
56	committees			
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other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)

## Introduction

Background and rationale	<a href="#">#6a</a>	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	<a href="#">#6b</a>	Explanation for choice of comparators	5
Objectives	<a href="#">#7</a>	Specific objectives or hypotheses	5
Trial design	<a href="#">#8</a>	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
<b>Methods:</b>			
Participants, interventions, and outcomes			
Study setting	<a href="#">#9</a>	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

1	Eligibility criteria	<a href="#">#10</a>	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
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11	Interventions:	<a href="#">#11a</a>	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6
12				
13	description			
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19	Interventions:	<a href="#">#11b</a>	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
20				
21	modifications			
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29	Interventions:	<a href="#">#11c</a>	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	7
30				
31	adherence			
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36	Interventions:	<a href="#">#11d</a>	Relevant concomitant care and interventions that are permitted or prohibited during the trial	N/A
37				
38	concomitant care			
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42	Outcomes	<a href="#">#12</a>	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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1	Participant timeline	<a href="#">#13</a>	Time schedule of enrolment, interventions (including any	8
2			run-ins and washouts), assessments, and visits for	
3			participants. A schematic diagram is highly	
4			recommended (see Figure)	
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11	Sample size	<a href="#">#14</a>	Estimated number of participants needed to achieve	8
12			study objectives and how it was determined, including	
13			clinical and statistical assumptions supporting any	
14			sample size calculations	
15				
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21	Recruitment	<a href="#">#15</a>	Strategies for achieving adequate participant enrolment	9
22			to reach target sample size	
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26	<b>Methods:</b>			
27				
28	<b>Assignment of</b>			
29	<b>interventions (for</b>			
30	<b>controlled trials)</b>			
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36	Allocation: sequence	<a href="#">#16a</a>	Method of generating the allocation sequence (eg,	6
37	generation		computer-generated random numbers), and list of any	
38			factors for stratification. To reduce predictability of a	
39			random sequence, details of any planned restriction (eg,	
40			blocking) should be provided in a separate document	
41			that is unavailable to those who enrol participants or	
42			assign interventions	
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53	Allocation	<a href="#">#16b</a>	Mechanism of implementing the allocation sequence	N/A
54	concealment		(eg, central telephone; sequentially numbered, opaque,	
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57				
58	mechanism			



1		sealed envelopes), describing any steps to conceal the	
2		sequence until interventions are assigned	
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6	Allocation:	<a href="#">#16c</a> Who will generate the allocation sequence, who will	6+9
7			
8	implementation	enrol participants, and who will assign participants to	
9		interventions	
10			
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12			
13	Blinding (masking)	<a href="#">#17a</a> Who will be blinded after assignment to interventions	6
14		(eg, trial participants, care providers, outcome	
15		assessors, data analysts), and how	
16			
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19			
20			
21	Blinding (masking):	<a href="#">#17b</a> If blinded, circumstances under which unblinding is	N/A
22		permissible, and procedure for revealing a participant's	
23	emergency	allocated intervention during the trial	
24			
25	unblinding		
26			
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29	<b>Methods: Data</b>		
30			
31	collection,		
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33	management, and		
34			
35	analysis		
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39	Data collection plan	<a href="#">#18a</a> Plans for assessment and collection of outcome,	9
40		baseline, and other trial data, including any related	
41		processes to promote data quality (eg, duplicate	
42		measurements, training of assessors) and a description	
43		of study instruments (eg, questionnaires, laboratory	
44		tests) along with their reliability and validity, if known.	
45			
46		Reference to where data collection forms can be found,	
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48		if not in the protocol	
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1	Data collection plan:	<a href="#">#18b</a>	Plans to promote participant retention and complete	N/A
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3	retention		follow-up, including list of any outcome data to be	
4			collected for participants who discontinue or deviate from	
5			intervention protocols	
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11	Data management	<a href="#">#19</a>	Plans for data entry, coding, security, and storage,	10
12			including any related processes to promote data quality	
13			(eg, double data entry; range checks for data values).	
14			Reference to where details of data management	
15			procedures can be found, if not in the protocol	
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23	Statistics: outcomes	<a href="#">#20a</a>	Statistical methods for analysing primary and secondary	10
24			outcomes. Reference to where other details of the	
25			statistical analysis plan can be found, if not in the	
26			protocol	
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33	Statistics: additional	<a href="#">#20b</a>	Methods for any additional analyses (eg, subgroup and	10
34	analyses		adjusted analyses)	
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39	Statistics: analysis	<a href="#">#20c</a>	Definition of analysis population relating to protocol non-	N/A
40	population and		adherence (eg, as randomised analysis), and any	
41	missing data		statistical methods to handle missing data (eg, multiple	
42			imputation)	
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48	<b>Methods: Monitoring</b>			
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51	Data monitoring:	<a href="#">#21a</a>	Composition of data monitoring committee (DMC);	11
52	formal committee		summary of its role and reporting structure; statement of	
53			whether it is independent from the sponsor and	
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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

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10	Data monitoring:	<a href="#">#21b</a>	Description of any interim analyses and stopping
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12	interim analysis		guidelines, including who will have access to these
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14			interim results and make the final decision to terminate
15			the trial
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20	Harms	<a href="#">#22</a>	Plans for collecting, assessing, reporting, and managing
21			
22			solicited and spontaneously reported adverse events
23			
24			and other unintended effects of trial interventions or trial
25			conduct
26			
27			
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30	Auditing	<a href="#">#23</a>	Frequency and procedures for auditing trial conduct, if
31			
32			any, and whether the process will be independent from
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34			investigators and the sponsor
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37			
38	<b>Ethics and</b>		
39			
40	<b>dissemination</b>		
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43	Research ethics	<a href="#">#24</a>	Plans for seeking research ethics committee /
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45	approval		institutional review board (REC / IRB) approval
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48	Protocol	<a href="#">#25</a>	Plans for communicating important protocol
49			
50	amendments		modifications (eg, changes to eligibility criteria,
51			outcomes, analyses) to relevant parties (eg,
52			investigators, REC / IRBs, trial participants, trial
53			registries, journals, regulators)
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1	Consent or assent	<a href="#">#26a</a>	Who will obtain informed consent or assent from	13
2			potential trial participants or authorised surrogates, and	
3			how (see Item 32)	
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9	Consent or assent:	<a href="#">#26b</a>	Additional consent provisions for collection and use of	N/A
10	ancillary studies		participant data and biological specimens in ancillary	
11			studies, if applicable	
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16	Confidentiality	<a href="#">#27</a>	How personal information about potential and enrolled	10
17			participants will be collected, shared, and maintained in	
18			order to protect confidentiality before, during, and after	
19			the trial	
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26	Declaration of	<a href="#">#28</a>	Financial and other competing interests for principal	14
27	interests		investigators for the overall trial and each study site	
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32	Data access	<a href="#">#29</a>	Statement of who will have access to the final trial	14
33			dataset, and disclosure of contractual agreements that	
34			limit such access for investigators	
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39	Ancillary and post	<a href="#">#30</a>	Provisions, if any, for ancillary and post-trial care, and for	13
40	trial care		compensation to those who suffer harm from trial	
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47	Dissemination policy:	<a href="#">#31a</a>	Plans for investigators and sponsor to communicate trial	14
48	trial results		results to participants, healthcare professionals, the	
49			public, and other relevant groups (eg, via publication,	
50			reporting in results databases, or other data sharing	
51			arrangements), including any publication restrictions	
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1 Dissemination policy: [#31b](#) Authorship eligibility guidelines and any intended use of 14  
 2 authorship professional writers  
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 6 Dissemination policy: [#31c](#) Plans, if any, for granting public access to the full 14  
 7 reproducible protocol, participant-level dataset, and statistical code  
 8 research  
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## 12 Appendices

13  
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 17 Informed consent [#32](#) Model consent form and other related documentation Appendix  
 18 materials given to participants and authorised surrogates  
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 23 Biological specimens [#33](#) Plans for collection, laboratory evaluation, and storage of N/A  
 24 biological specimens for genetic or molecular analysis in  
 25 the current trial and for future use in ancillary studies, if  
 26 applicable  
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 33 None The SPIRIT Explanation and Elaboration paper is distributed under the terms of the Creative  
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# 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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15 Protocol version: 5.0, 12-NOV-21

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18  
19 Keywords: Randomized controlled trial, Gram-negative bacteremia, bloodstream infection, treatment duration, antibiotic  
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21 stewardship, short-course therapy

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24 Randomized controlled trial, Gram-negative bacteremia, bloodstream infection, Treatment duration,  
25 Word count: 3816

## 26 27 28 29 **ABSTRACT**

30  
31 **Introduction:** Prolonged use of antibiotics is closely related to antibiotic-associated infections,  
32  
33 antimicrobial resistance, and adverse drug events. The optimal duration of antibiotic treatment for  
34  
35 Gram-negative bacteremia (GNB) with a urinary tract source of infection is poorly defined.

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38 **Methods and analysis:** Investigator initiated multicenter, non-blinded, non-inferiority randomized  
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40 controlled trial with two parallel treatment arms. One arm will receive shortened antibiotic treatment  
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42 of five days and the other arm will receive antibiotic treatment of seven days or longer.  
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44 Randomization will occur in equal proportion (1:1) no later than day five of effective antibiotic  
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46 treatment as determined by antibiogram. Immunosuppressed patients and those with GNB due to  
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48 non-fermenting bacilli (*Acinetobacter* spp., *Pseudomonas* spp.), *Brucella* spp., *Fusobacterium* spp.  
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50 or polymicrobial growth are ineligible.  
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55 The primary endpoint is 90-day survival without clinical or microbiological failure to treatment.

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58 Secondary endpoints include all-cause mortality, total duration of antibiotic treatment, hospital  
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4 readmission, and *Clostridioides difficile* infection. Interim safety analysis will be performed after the  
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6 recruitment of every 100 patients. Given an event rate of 12%, a non-inferiority margin of 10%, and  
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8 90% power, the required sample size to determine non-inferiority is 380 patients. Analyses will be  
9  
10 performed on both intention-to-treat and per-protocol populations.  
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14 **Ethics and dissemination:** The study is approved by the Danish Regional Committee on Health  
15  
16 Research (H-19085920) and the Danish Medicines Agency (2019-003282-17). The results of the  
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18 main trial and each of the secondary endpoints will be submitted for publication in a peer-reviewed  
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20 journal.  
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24 **Trial registration:** ClinicalTrials.Gov: NCT04291768. Registered on the 24<sup>th</sup> of February 2020.  
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## 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.

Peer review only

## 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]

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

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

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

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

### 41 **Trial design and randomization**

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43 An investigator-initiated multicenter, non-blinded, non-inferiority randomized controlled trial with two  
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45 parallel treatment arms. One arm will receive shortened antibiotic treatment guided by clinical  
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47 stability criteria (intervention group) and the other arm will receive standard antibiotic treatment  
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49 (control group). As the treatment duration relies on continuous evaluation of clinical stability criteria  
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51 of the participants, blinding of study personnel and participants is not practicable. The design of the  
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4 study also strives to be as close to standard clinical practice as possible. Therefore, study  
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6 investigators, trial participants, or treating physicians will not be blinded to treatment allocation.  
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11 Confirmation of study eligibility will be performed by entering key variables into a secure web-based  
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13 program (REDCap) with subsequent automatic patient randomization in the two parallel arms (ratio  
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15 1:1) no later than day five after initiation of microbiologically effective empiric antibiotic treatment.  
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19 The randomization list will be generated centrally in random blocs and stratified according to hospital  
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21 and etiology. The randomization key will be stored in a locked and secure environment at  
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23 Copenhagen University Hospital – Hvidovre Hospital.  
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## 26 27 28 **Study setting**

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30 The following twelve hospitals, representing all five regions of Denmark, will participate in the study:  
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33 Copenhagen University Hospital – Amager and Hvidovre, Copenhagen University Hospital –  
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35 Rigshospitalet, Copenhagen University Hospital – Bispebjerg and Frederiksberg, Copenhagen  
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37 University Hospital – Herlev and Gentofte, Copenhagen University Hospital – North Zealand,  
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39 Zealand University Hospital – Roskilde, Odense University Hospital, Kolding Hospital, Silkeborg  
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41 Hospital, Herning Hospital, Aarhus University Hospital, and Aalborg University Hospital.  
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## 48 **Eligibility criteria**

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50 Inclusion and exclusion criteria are listed in Table 1.  
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53 **Table 1.** *Inclusion and exclusion criteria*

Inclusion criteria	Exclusion criteria
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<ul style="list-style-type: none"> <li>• Age <math>\geq 18</math> years</li> <li>• Blood culture positive for Gram-negative bacteria</li> <li>• Evidence of urinary tract source of infection (positive urine culture with the same bacteria as in the blood culture <u>or</u> at least one clinical symptom compatible with urinary tract infection)</li> <li>• Antibiotic treatment with antimicrobial activity to Gram-negative bacteria administered within 12 hours of first blood culture</li> <li>• Temperature <math>\leq 37.8^{\circ}\text{C}</math> at randomization</li> <li>• Clinically stable at randomization (systolic blood pressure <math>&gt; 90</math> mm Hg, heart rate <math>&lt; 100</math> beats/min., respiratory rate <math>&lt; 24</math>/minute, peripheral oxygen saturation <math>&gt; 90\%</math>)</li> <li>• Oral and written informed consent</li> </ul>	<ul style="list-style-type: none"> <li>• Antibiotic treatment (<math>&gt; 2</math> days) with antimicrobial activity to Gram-negative bacteria within 14 days of inclusion</li> <li>• Gram-negative bacteremia within 30 days of blood culture</li> <li>• Immunosuppression <ul style="list-style-type: none"> <li>○ Untreated HIV-infection</li> <li>○ Neutropenia (absolute neutrophil count <math>&lt; 1.0 \times 10^9/l</math>)</li> <li>○ Untreated terminal cancer</li> <li>○ Receiving immunosuppressive agents (ATC-code L04A)</li> <li>○ Corticosteroid treatment (<math>\geq 20</math> mg/day prednisone or the equivalent for <math>&gt; 14</math> days) within the last 30 days</li> <li>○ Chemotherapy within the last 30 days</li> <li>○ Immunosuppressed after solid organ transplantation</li> <li>○ Asplenia</li> </ul> </li> <li>• Polymicrobial growth in blood culture</li> <li>• Bacteremia with non-fermenting Gram-negative bacteria (<i>Acinetobacter</i> spp, <i>Burkholderia</i> spp, <i>Pseudomonas</i> spp), <i>Brucella</i> spp, or <i>Fusobacterium</i> spp</li> </ul>
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|--|---|
|  | <ul style="list-style-type: none"> <li>• Failure to remove the source of infection within 72 hours of first blood culture (e.g. change of indwelling catheter)</li> <li>• Pregnancy or breastfeeding</li> </ul> |
|--|---|

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	Administratio n <sup>1</sup>	Standard dose <sup>1</sup>	Frequency <sup>1</sup>	Dose adjustment <sup>1</sup>
<i>Penicillins</i>				
Piperacillin/Tazobactam	IV	4 g/0.5 g	Every 6 or 8 hours	Renal impairment and weight
Ampicillin <sup>2</sup>	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
<i>Cephalosporins</i>				
Cefuroxime <sup>3</sup>	IV	1.5 g	Every 6 hours	Renal impairment and weight
Cefotaxime <sup>3</sup>	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 Every 24 hours	Renal and hepatic impairment and weight
<i>Carbapenems</i>				
Meropenem	IV	1 g	Every 8 hours	Renal impairment and weight

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Ertapenem	IV	1 g	Every 24 hours	-
<i>Aminoglycosides</i>				
Gentamicin <sup>2,3/</sup> tobramycin	IV	5-7 mg/kg	Every 24 hours	Renal impairment and weight
<i>Fluoroquinolone</i>				
Ciprofloxacin	IV	400 mg	Every 12 hours	Renal impairment and weight

<sup>1</sup>Standard recommendations, <sup>2</sup>Combination therapy of ampicillin and gentamicin, <sup>3</sup>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 <sup>1</sup>	Standard dose <sup>1</sup>	Frequency <sup>1</sup>	Dose adjustment <sup>1</sup>
<i>Penicillin</i>				
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
<i>Cephalosporin</i>				
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
<i>Carbapenem</i>				
Meropenem	IV	1 g	Every 8 hours	Renal impairment and weight
Ertapenem	IV	1 g	Every 24 hours	-
<i>Aminoglycoside</i>				
Gentamicin/tobramycin	IV	5-7 mg/kg	Every 24 hours	Renal impairment and weight
<i>Fluoroquinolone</i>				
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

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4 Sulfamethoxazole/Trimethoprim

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6 PO

800 mg/160 mg

Every 12 hours

Renal impairment and weight

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10 <sup>1</sup>Standard recommendation

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13  
14 Antibiotics will be administered to participants by clinical staff as in usual clinical care during  
15 hospitalization. Practical procedures related to antibiotic treatment, including labeling of applied  
16 drugs, will follow normal local instructions while the participant is hospitalized. If participants are  
17 discharged before the end of therapy, the exact amount of remaining antibiotics will be delivered  
18 from the hospital in the original packaging supplied with the additional label. Trained personnel will  
19 perform the additional labeling.  
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31 Treatment adherence is evaluated by checking medicine administration records of inpatients or by  
32 thoroughly interviewing outpatients about their consumption at the planned telephone interview on  
33 day 14. Outpatients will be instructed to document self-administered antibiotic treatment at home to  
34 ensure a more accurate measurement of adherence following hospital discharge. Protocol violations  
35 will be reported if patients are assessed to be non-compliant (received <80% of scheduled doses).  
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## 45 Outcomes

### 46 Primary outcome

47  
48 90-day survival without clinical or microbiological failure to treatment as defined:

- 49 1. All-cause mortality from the day of randomization until day 90
- 50 2. Microbiological failure: Recurrent bacteremia due to the same microorganism as verified by  
51 sequence analysis occurring after day 5 of antibiotic treatment and until day 90  
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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
- Distant complications of initial infection, defined by the growth of the same bacteria as in the initial bacteremia (e.g. endocarditis, meningitis)
  - 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  $\geq 3$  as described elsewhere  
([https://ctep.cancer.gov/protocoldevelopment/electronic\\_applications/ctc.htm](https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm))
- Acute kidney injury
- *Clostridioides s difficile* infection
- Infection with multidrug-resistance organism

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4 A single bacteremic episode was defined as including all positive blood cultures with the same  
5  
6 organism within a five-day period, therefore recurrence was defined as occurring after five days of  
7  
8 antibiotic treatment and until day 90.  
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10

### 11 12 13 14 **Participant timeline**

15  
16 After an initial observation and information period, the participants will be included in the study and  
17  
18 randomized no later than day five after the initiation of appropriate empiric antibiotic treatment.  
19  
20 Participants will be followed for 90 days. On day 14 (12-16) of follow-up, participants will be  
21  
22 scheduled for blood sample collection and standardized telephone interview. On day 90 (83-97) of  
23  
24 follow-up, participants will be scheduled for a final standardized telephone interview. The study  
25  
26 flowchart depicts the inclusion, randomization, allocation, follow up and analysis of participants  
27  
28 throughout the study, see Figure 1 '*Flow chart on study level*'. The participant timeline is illustrated  
29  
30 in Figure 2 '*Flow chart on participant level*'.  
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### 38 **Sample size**

39  
40 Based on a similar previous study, short-term mortality is expected to be approximately 12% in both  
41  
42 treatment arms. Failure is expected to be 8% in both study arms[19]. Because individuals who are  
43  
44 not stable by day five, who have complicated infections, who have a polymicrobial infection or  
45  
46 infection with *Pseudomonas* spp., *Brucella* spp., and *Fusobacterium* spp. are not eligible, we  
47  
48 anticipate these rates to be lower at 8% and 4%, respectively. This corresponds to an estimated  
49  
50 event rate for the primary outcome of approximately 12%, equivalent to a 90-day survival without  
51  
52 clinical or microbiological failure to treatment or relapse of 88 % in both treatment arms.  
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4 Non-inferiority is defined as an absolute risk difference or margin in the primary endpoint of up to  
5  
6 10%, as recommended by the European Medicines Agency.[26] Given an  $\alpha$  of 5% and a  $\beta$  of 90%  
7  
8 then 362 randomized individuals are required to be sure that the lower limit of a one-sided 95%  
9  
10 confidence interval will exclude a difference in favor of the longer course of antibiotics of more than  
11  
12 10%. Allowing for a dropout rate of 5%, 380 individuals will be included.  
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18  
19 A sample size re-estimation (SSR) will be considered at the first planned interim analysis. If the  
20  
21 overall event rate falls outside the expected event rate of 12%, an SSR based upon a blinded review  
22  
23 of overall data (i.e. without knowledge of the group-specific event rates) will be performed. If the  
24  
25 overall event rate is lower than expected, then the final sample size will be reduced, using the original  
26  
27 sample size formula, and replacing the initial estimate with the observed rate. The non-inferiority  
28  
29 margin may be reduced to ensure an appropriate margin relative to the event rate. If the overall event  
30  
31 rate is higher than expected, the sample size will be increased correspondingly. Any sample size  
32  
33 adjustment will be reported to the regulatory authorities as a protocol amendment.  
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## 40 **Recruitment**

41  
42 Investigators and treating physicians at participating sites will identify patients eligible for the trial  
43  
44 during days one through four after initiation of empiric antibiotic treatment. Cases are identified by  
45  
46 participating Departments of Clinical Microbiology and Infectious Diseases at each center. All  
47  
48 participants are hospitalized at enrollment.  
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52  
53 The principal investigator will handle questions concerning the recruitment or enrolment of  
54  
55 participants, while the study is running.  
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## 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 <sup>1</sup>		STUDY PERIOD <sup>2</sup>	
	Day 1	Day 2-5	Day 14 (12-16)	Day 90 (83-97)

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

<sup>1</sup>From day 1 of efficacious empiric antibiotic treatment to inclusion

<sup>2</sup>From inclusion to the end of the trial on day 90

<sup>3</sup>Age, gender, tobacco use, alcohol consumption, medication, medical history, nursing home residency, and activities of daily living

<sup>4</sup>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

<sup>5</sup>Blood pressure, heart rate, temperature, respiratory rate, peripheral oxygen saturation

<sup>6</sup>Haemoglobin, leukocytes (WBCs), platelet count, CRP, creatinine, urea, sodium, potassium, bilirubin, alanine aminotransferase, glucose

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<sup>7</sup>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)<sup>31</sup>

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

<sup>9</sup>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

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4 Descriptive statistics will be presented as frequency tables, means with standard deviations, or  
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7 medians with interquartile ranges.  
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11 Both intention-to-treat (ITT) and per-protocol (PP) analyses will be performed. Intention-to-treat  
12  
13 analysis will comprise all participants including dropouts. Categorical variables will be analyzed with  
14  
15  $\chi^2$ -test or Fisher's exact test. Continuous variables will be subject to Student's t-test or Wilcoxon rank  
16  
17  
18 sum test.  
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23 Subgroup-analyses are planned for disease severity, antibiotic group, resistant pathogens, and  
24  
25 investigating center. Resistant pathogens are defined as extended-spectrum beta-lactamase (ESBL)  
26  
27 producing or carbapenemase-producing Enterobacteriaceae, or pathogens with lack of susceptibility  
28  
29 to minimum one agent in three or more classes of antibiotic.  
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35 Non-inferiority plots will be performed on the primary outcome for both ITT and PP analyses.  
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40 For all statistical analyses except for the non-inferiority analysis, a two-sided p-value <0.05 is  
41  
42 considered statistically significant.  
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47 The Statistical Analysis Plan is available in Supplemental Material.  
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## 50 51 52 **Data monitoring**

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

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4 Participants will be thoroughly asked if they have experienced any adverse event at inclusion and  
5  
6 the planned follow-up by phone on days 14 and 90. Adverse events will be registered in predefined  
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8 CRFs. All adverse events will be followed until they have abated, or until a stable situation has been  
9  
10 reached.  
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16 All adverse events must be evaluated by investigators and sponsor to determine possible causal  
17  
18 association with the antibiotic treatment. At study termination, a final report of registered events in  
19  
20 the CRFs will be sent to the Danish Medicines Agency and the Health Research Ethics Committee  
21  
22 of the Capital Region of Denmark. Serious adverse reactions, suspected unexpected serious  
23  
24 adverse reactions, and information on the general safety of the participants will be listed in an annual  
25  
26 safety report to the Danish Medicines Agency and the regional Health Research Ethics Committee.  
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## 30 31 32 33 **DISCUSSION**

34  
35 The results of this study may have major implications on antibiotic use in Gram-negative bacteremia  
36  
37 with urinary tract infection as source of infection. Regardless of the results, the study will provide  
38  
39 valuable information about current treatment practices by either validating or revising them in light of  
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41 the scientific evidence. To the best of our knowledge, this is the first study to assess the safety and  
42  
43 efficacy of five days of treatment in Gram-negative bacteremia guided by clinical stability criteria. We  
44  
45 chose 90-day survival without microbiological and clinical failure as the primary endpoint, as we  
46  
47 consider failure and mortality to be the most important measures for clinical safety.  
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54 As a potential disadvantage related to shortened antibiotic duration would be the risk of treatment  
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56 failure, it is, therefore, crucial to carefully select study participants when considering both efficacy  
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4 and safety. Accordingly, the inclusion and exclusion criteria of the study were thoroughly based on  
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6 available data on risk factors related to clinical outcomes. Relying on clinical stability criteria in  
7  
8 shortening antibiotic treatment is partly based on a large randomized controlled trial showing that  
9  
10 shortened antibiotic treatment against Gram-negative bacteremia is non-inferior to longer antibiotic  
11  
12 treatment in patients that reached clinical stability within seven days of treatment.[19] Treatment  
13  
14 failure in patients hospitalized with Gram-negative bacteremia has been shown to correlate with  
15  
16 initial disease severity, e.g. by Pitt bacteremia score, end-stage liver disease, and  
17  
18 immunosuppression.[17] According to protocol, all participants are scheduled for a blood test on day  
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20 14 and a standardized telephone interview on day 14, and the last day of follow-up, on day 90, which  
21  
22 will ensure early detection of potential treatment failure.  
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31 A limitation to this study is that our strict eligibility criteria will possibly limit the generalizability of the  
32  
33 results to all patient groups, as only patients with uncomplicated disease are included.  
34  
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37

38 If shortened antibiotic duration in patients hospitalized with Gram-negative bacteremia can be proven  
39  
40 to be non-inferior to standard antibiotic treatment, it would likely relieve antibiotic selective pressure  
41  
42 and thereby lower the development of bacterial resistance.[3,6,11,12] From the perspective of the  
43  
44 participants, one might expect fewer side effects and better treatment adherence, as prolonged  
45  
46 antibiotic use has been associated with an increased risk of side effects.[9] On a community level,  
47  
48 this change would lead to a reduction in overall health care costs, as shortened antibiotic duration  
49  
50 would result in a decrease in total antibiotic consumption and thereby length of hospital stay.[29–31]  
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52 As Gram-negative bacteria are accountable for great proportions of bacteremia and thereby  
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54 antibiotic prescription, this decrease could be quite significant from a national perspective.  
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## 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, 18/02/2020	Approved for study start
Version 4, 22/09/2020	<ul style="list-style-type: none"> <li>• Exclusion criteria are added:               <ul style="list-style-type: none"> <li>○ Gram-negative bacteremia within the past 30 days.</li> <li>○ Antibiotic treatment &gt;1 day with antimicrobial activity to Gram-negative bacteria within past 14 days.</li> <li>○ Untreated terminal cancer.</li> </ul> </li> <li>• Exclusion criteria regarding chemotherapy and immunosuppression are further specified.</li> <li>• Temperature limit for afebrile is changed from 38.0°C to 37.8°C.</li> <li>• Change of site investigator at Copenhagen University Hospital – Gentofte.</li> </ul>
Version 5, 12/11/2021	<ul style="list-style-type: none"> <li>• Study period is extended with two years.</li> </ul>

	<ul style="list-style-type: none"><li>• Copenhagen University Hospital – Bispebjerg and Frederiksberg is included as study site.</li><li>• Exclusion criterium regarding antibiotic treatment prior to inclusion is changed from &gt;1 day to &gt;2 days.</li></ul>
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### Access to data

The study is registered at [www.clinicaltrials.gov](http://www.clinicaltrials.gov) before initiation (ClinicalTrials.Gov: NCT04291768, registered on the 24<sup>th</sup> 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

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3  
4 Dickinson, Janssen and Astra Zeneca; outside the submitted work. The remaining authors have no  
5  
6 conflicts of interest to declare.  
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## 12 **Author contributions**

13  
14  
15 TB is the sponsor of the study. ST is the coordinating principal investigator. TB conceived the  
16  
17 research question of the study. TB and ST obtained the funding for the study. TB, ST, SBI, LTU and  
18  
19 CØ participated in the design of the study. ST drafted the study protocol. TB, ST, SBI, LTU, BL, IJ,  
20  
21 SL, CØ, PR, KK, and AK contributed to the implementation of the study and revised the protocol  
22  
23 critically for important intellectual content. All authors read and approved the final manuscript.  
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31  
32 Haakon Sandholt for statistical support.  
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39  
40 The study is partly financially supported by a grant from Hvidovre Hospital Strategic Research Fund,  
41  
42 Danish Regions Medicine Grant, grant number EMN-2019-01055, and Capital Region of Denmark  
43  
44 Research Fund, grant number A6688. Additional federal funding will be sought. Study participants  
45  
46 and the Research Ethics Committee of the Capital Region of Denmark will be informed if additional  
47  
48 funding has been granted. Sponsor and investigators are independent of economic or competing  
49  
50 interests. Participants will not be financially reimbursed. Results from the study are only for scientific  
51  
52 and public use and have no commercial interest.  
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*Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* Published Online First: 11 December 2018. doi:10.1093/cid/ciy1054

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4 **Figure 1.** *Flow chart on study level*  
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9 **Figure 2.** *Flowchart on participant level*  
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For peer review only



**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  $\geq 18$  years
- Blood culture positive for Gram-negative bacteria
- Evidence of urinary tract source of infection
- Appropriate empiric antibiotic administered 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:**

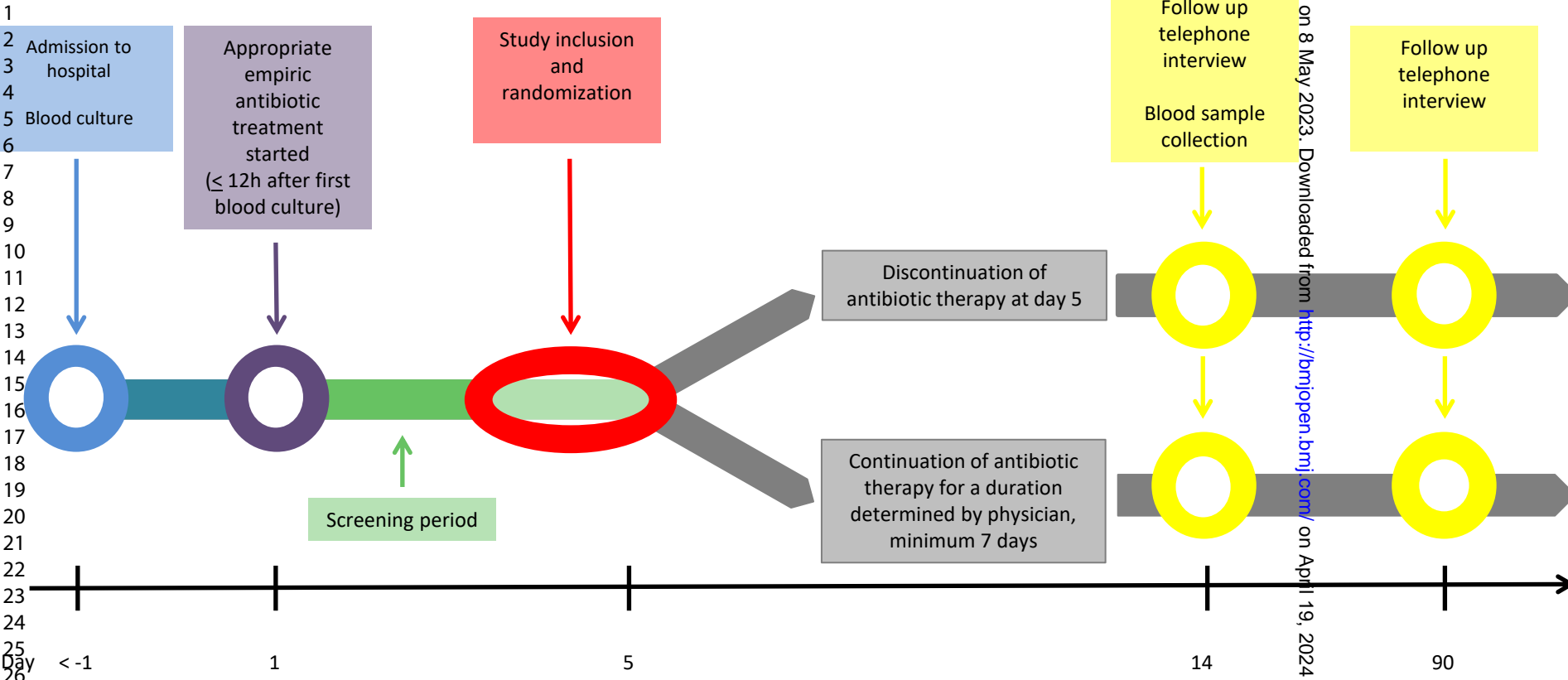
- Structured telephone interview
- Blood sample collection

**Day 90:**

- Structured telephone interview
- Vital status
- Additional antibiotic treatment
- Hospital readmission

**Day 90:**

- Structured telephone interview
- Vital status
- Additional antibiotic treatment
- Hospital readmission



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Eudra-CT no.: 2019-003282-17

GNB5

# 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  


Statistician

6/2-20  


Principal investigator

6/2-20  


Sponsor

Eudra-CT no.: 2019-003282-17

GNB5

## **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  $\geq 7$  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

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GNBS

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

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population. Minor procedural variations (e.g. failure to collect additional blood samples at inclusion) will not preclude 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  $\geq 3$
- Acute kidney injury
- *Clostridium 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.

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GNB5

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 SPIRIT reporting 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

		Page
	Reporting Item	Number
<b>Administrative information</b>		
Title	<a href="#">#1</a> Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1

1	Trial registration	<a href="#">#2a</a>	Trial identifier and registry name. If not yet registered,	1
2			name of intended registry	
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6	Trial registration:	<a href="#">#2b</a>	All items from the World Health Organization Trial	N/A
7	data set		Registration Data Set	
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12	Protocol version	<a href="#">#3</a>	Date and version identifier	1
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14				
15	Funding	<a href="#">#4</a>	Sources and types of financial, material, and other	13
16			support	
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19				
20	Roles and	<a href="#">#5a</a>	Names, affiliations, and roles of protocol contributors	1+14
21	responsibilities:			
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23	contributorship			
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28	Roles and	<a href="#">#5b</a>	Name and contact information for the trial sponsor	1+14
29	responsibilities:			
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31	sponsor contact			
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33	information			
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38	Roles and	<a href="#">#5c</a>	Role of study sponsor and funders, if any, in study	14
39	responsibilities:		design; collection, management, analysis, and	
40			interpretation of data; writing of the report; and the	
41	sponsor and funder		decision to submit the report for publication, including	
42			whether they will have ultimate authority over any of	
43			these activities	
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52	Roles and	<a href="#">#5d</a>	Composition, roles, and responsibilities of the	14
53	responsibilities:		coordinating centre, steering committee, endpoint	
54			adjudication committee, data management team, and	
55	committees			
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other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)

## Introduction

Background and rationale	<a href="#">#6a</a>	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	<a href="#">#6b</a>	Explanation for choice of comparators	5
Objectives	<a href="#">#7</a>	Specific objectives or hypotheses	5
Trial design	<a href="#">#8</a>	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
<b>Methods:</b>			
<b>Participants, interventions, and outcomes</b>			
Study setting	<a href="#">#9</a>	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

1	Eligibility criteria	<a href="#">#10</a>	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
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11	Interventions:	<a href="#">#11a</a>	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6
12				
13	description			
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19	Interventions:	<a href="#">#11b</a>	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
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21	modifications			
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29	Interventions:	<a href="#">#11c</a>	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	7
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31	adherence			
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36	Interventions:	<a href="#">#11d</a>	Relevant concomitant care and interventions that are permitted or prohibited during the trial	N/A
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38	concomitant care			
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42	Outcomes	<a href="#">#12</a>	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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1	Participant timeline	<a href="#">#13</a>	Time schedule of enrolment, interventions (including any	8
2			run-ins and washouts), assessments, and visits for	
3			participants. A schematic diagram is highly	
4			recommended (see Figure)	
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11	Sample size	<a href="#">#14</a>	Estimated number of participants needed to achieve	8
12			study objectives and how it was determined, including	
13			clinical and statistical assumptions supporting any	
14			sample size calculations	
15				
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21	Recruitment	<a href="#">#15</a>	Strategies for achieving adequate participant enrolment	9
22			to reach target sample size	
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26	<b>Methods:</b>			
27				
28	<b>Assignment of</b>			
29	<b>interventions (for</b>			
30	<b>controlled trials)</b>			
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36	Allocation: sequence	<a href="#">#16a</a>	Method of generating the allocation sequence (eg,	6
37	generation		computer-generated random numbers), and list of any	
38			factors for stratification. To reduce predictability of a	
39			random sequence, details of any planned restriction (eg,	
40			blocking) should be provided in a separate document	
41			that is unavailable to those who enrol participants or	
42			assign interventions	
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53	Allocation	<a href="#">#16b</a>	Mechanism of implementing the allocation sequence	N/A
54	concealment		(eg, central telephone; sequentially numbered, opaque,	
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57				
58	mechanism			

sealed envelopes), describing any steps to conceal the sequence until interventions are assigned

Allocation: [#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): [#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

1	Data collection plan:	<a href="#">#18b</a>	Plans to promote participant retention and complete	N/A
2				
3	retention		follow-up, including list of any outcome data to be	
4			collected for participants who discontinue or deviate from	
5			intervention protocols	
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11	Data management	<a href="#">#19</a>	Plans for data entry, coding, security, and storage,	10
12			including any related processes to promote data quality	
13			(eg, double data entry; range checks for data values).	
14			Reference to where details of data management	
15			procedures can be found, if not in the protocol	
16				
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23	Statistics: outcomes	<a href="#">#20a</a>	Statistical methods for analysing primary and secondary	10
24			outcomes. Reference to where other details of the	
25			statistical analysis plan can be found, if not in the	
26			protocol	
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33	Statistics: additional	<a href="#">#20b</a>	Methods for any additional analyses (eg, subgroup and	10
34	analyses		adjusted analyses)	
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39	Statistics: analysis	<a href="#">#20c</a>	Definition of analysis population relating to protocol non-	N/A
40	population and		adherence (eg, as randomised analysis), and any	
41	missing data		statistical methods to handle missing data (eg, multiple	
42			imputation)	
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48	<b>Methods: Monitoring</b>			
49				
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51	Data monitoring:	<a href="#">#21a</a>	Composition of data monitoring committee (DMC);	11
52	formal committee		summary of its role and reporting structure; statement of	
53			whether it is independent from the sponsor and	
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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

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10	Data monitoring:	<a href="#">#21b</a>	Description of any interim analyses and stopping
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12	interim analysis		guidelines, including who will have access to these
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14			interim results and make the final decision to terminate
15			the trial
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20	Harms	<a href="#">#22</a>	Plans for collecting, assessing, reporting, and managing
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22			solicited and spontaneously reported adverse events
23			
24			and other unintended effects of trial interventions or trial
25			conduct
26			
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30	Auditing	<a href="#">#23</a>	Frequency and procedures for auditing trial conduct, if
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32			any, and whether the process will be independent from
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34			investigators and the sponsor
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38	<b>Ethics and</b>		
39			
40	<b>dissemination</b>		
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42			
43	Research ethics	<a href="#">#24</a>	Plans for seeking research ethics committee /
44			
45	approval		institutional review board (REC / IRB) approval
46			
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48	Protocol	<a href="#">#25</a>	Plans for communicating important protocol
49			
50	amendments		modifications (eg, changes to eligibility criteria,
51			outcomes, analyses) to relevant parties (eg,
52			investigators, REC / IRBs, trial participants, trial
53			registries, journals, regulators)
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1	Consent or assent	<a href="#">#26a</a>	Who will obtain informed consent or assent from	13
2			potential trial participants or authorised surrogates, and	
3			how (see Item 32)	
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9	Consent or assent:	<a href="#">#26b</a>	Additional consent provisions for collection and use of	N/A
10	ancillary studies		participant data and biological specimens in ancillary	
11			studies, if applicable	
12				
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16	Confidentiality	<a href="#">#27</a>	How personal information about potential and enrolled	10
17			participants will be collected, shared, and maintained in	
18			order to protect confidentiality before, during, and after	
19			the trial	
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26	Declaration of	<a href="#">#28</a>	Financial and other competing interests for principal	14
27	interests		investigators for the overall trial and each study site	
28				
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32	Data access	<a href="#">#29</a>	Statement of who will have access to the final trial	14
33			dataset, and disclosure of contractual agreements that	
34			limit such access for investigators	
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39	Ancillary and post	<a href="#">#30</a>	Provisions, if any, for ancillary and post-trial care, and for	13
40	trial care		compensation to those who suffer harm from trial	
41			participation	
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47	Dissemination policy:	<a href="#">#31a</a>	Plans for investigators and sponsor to communicate trial	14
48	trial results		results to participants, healthcare professionals, the	
49			public, and other relevant groups (eg, via publication,	
50			reporting in results databases, or other data sharing	
51			arrangements), including any publication restrictions	
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1 Dissemination policy: [#31b](#) Authorship eligibility guidelines and any intended use of 14  
 2 authorship professional writers  
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6 Dissemination policy: [#31c](#) Plans, if any, for granting public access to the full 14  
 7 reproducible protocol, participant-level dataset, and statistical code  
 8 research  
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## 13 Appendices

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 16  
 17 Informed consent [#32](#) Model consent form and other related documentation Appendix  
 18 materials given to participants and authorised surrogates  
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 23 Biological specimens [#33](#) Plans for collection, laboratory evaluation, and storage of N/A  
 24 biological specimens for genetic or molecular analysis in  
 25 the current trial and for future use in ancillary studies, if  
 26 applicable  
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 33 None The SPIRIT Explanation and Elaboration paper is distributed under the terms of the Creative  
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