Supplementary Online Content

von Dach E, Albrich WC, Brunel AS, et al. Effect of C-reactive protein–guided antibiotic treatment duration, 7-day treatment, or 14-day treatment on 30-day clinical failure rate in patients with uncomplicated gram-negative bacteremia. *JAMA*. doi:10.1001/jama.2020.6348

Supplement 2. Statistical analysis plan

This supplementary material has been provided by the authors to give readers additional information about their work.

STATISTICAL ANALYSIS PLAN
Protocol 2017-00108

The PIRATE PROJECT: a Point-of-care, Informatics-based Randomized, controlled trial for decreasing over-utilization of Antibiotic ThErapy in Gram-negative Bacteremia

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Document history

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Table of Contents

1 Introduction & data preparation

This document describes the statistical analysis plan (SAP) for the multicenter non-inferiority trial entitled "The PIRATE PROJECT: a Point-of-care, Informatics-based Randomized, controlled trial for decreasing over-utilization of Antibiotic ThErapy in Gram-negative Bacteremia." All study outcome measures and analysis populations are reviewed in Sections 2 and 3, respectively. Please refer to the study protocol (v1.5, 16.07.2018) for all other details on study design. Of note, the non-inferiority hypothesis pertains solely to the primary outcome of clinical response and not to any secondary outcomes. Analysis plans for nested studies (PIRATE RESISTANCE, PIRATE Endurance, etc.) are not included here.

Data source. The information collected in the case report form (CRF) has been transferred into a dedicated database (SecuTrial). Only data stored within SecuTrial will be analyzed.

Database lock. No data will be exported for analysis until the data have been validated by study investigators and database managers, and the database locked by the study's data managers in Geneva.

Data-analyst blinding. As described in the study protocol, all data analyses will be conducted in a blinded fashion. Through the aid of data managers, data exports from Secutrial will include "scrambled" (recoded) patient study numbers to avoid recognition of a patient by study investigators, and individual treatment assignments will be coded to ensure masking of group treatment assignment.

2 Study outcomes

The following outcomes are found in the trial protocol.

Primary outcome. The primary outcome of the PIRATE trial is the *clinical failure rate in all arms through day 30*. Clinical failure is defined by the presence of at least one of the following:

- Relapse: a recurrent bacteremia due to the same bacterium occurring from the day of treatment cessation and through day 30
- Local suppurative complication that was not present at infection onset (e.g., renal abscess in pyelonephritis, empyema in pneumonia)
- Distant complications of the initial infection, defined by growth of the same bacterium causing the initial bacteremia (as determined by antibiotic susceptibility profiling)
- The restarting of Gram-negative-directed antibiotic therapy after its initial discontinuation due to clinical worsening suspected to be due to the initial infecting organism and for which there is no alternate diagnosis/pathogen suspected
- Death due to any cause through day 30

Secondary outcomes are as follows:

- The incidence of clinical failure through days 60 and 90 as defined in the primary outcome
- All-cause mortality through days 30, 60 and 90
- The total number of antibiotic days
- The incidence of antibiotic-related adverse events through day 90 (including *Clostridium difficile* infection)
- The incidence of the emergence of bacterial resistance in those with recurrence
- The number of patients in each arm whose assigned antibiotic duration was "overridden" by physicians in the absence of clinical failure (and the reasons for these deviations)

- Cost-effectiveness analyses (CEA) using quality-adjusted life-years (QALY) as the outcome measure
- Length of hospital stay (acute-care)

In addition to the protocol-defined outcomes, we will examine

- The clinical failure rate in all arms in the **30 days after antibiotic therapy cessation**
- The clinical failure rate in all arms in the **60 days after antibiotic therapy cessation**
- The clinical failure rate in all arms in the **90 days after antibiotic therapy cessation**
- Total number of rehabilitation/long-term-care-facility (LTCF) days by days 30, 60 and 90
- The association, if any, between the speed and/or amplitude of reductions in CRP with favorable clinical outcome at 30, 60 and 90 days/good prognosis

Subgroup analyses for the above outcomes will be performed by:

- Main causative organisms (*Escherichia coli*, *Klebsiella* spp., *Proteus* spp., non-enterobacteriaceae, anaerobes)
- Anatomic focus of primary infection (involved organ systems)
- Infection acquisition type (community-acquired, healthcare-associated, nosocomial); see definitions in protocol
- Center and type of healthcare setting (acute-care versus geriatric hospital/LTCF)
- Antibiotic regimens including single vs. combination therapy, and de-escalation
- Patients with only intravenous vs. intravenous followed by oral antibiotics
- Patients with effective appropriate empiric antibiotics (defined as susceptible pathogen) vs. patients with inappropriate empiric antibiotics
- Proportion of patients with multiresistant organism(s) at inclusion (see definition below)
- Proportion of patients with persistent bacteremia (over 24 h or 48 h)
- Patients without comorbidities vs. those with 1-3 comorbidities, and those with ≥3 comorbidities
- Patients with immunodeficiency (i.e. as long as no exclusion criterion fulfilled)
- Empiric therapy with different antibiotic classes (penicillins, penicillin/β-lactamase-inhibitor, cephalosporin, carbapenem, non-β-lactam)
- Empiric therapy with anaerobic coverage vs. no anaerobic coverage
- Patients who did not fulfill the per-protocol criteria:
    - Patients in the CRP group following the discontinuation algorithm according to protocol versus those with incomplete CRP follow-up and deviations from the algorithm

Potential risk factors for clinical failure will be determined, such as age, gender, antibiotic choice, anatomic focus of primary infection, comorbidity status, infection acquisition type (community vs. nosocomial), kinetics of the CRP response (see above), center effect and severity of illness at the time of diagnosis.

3 Statistical methods

3.1 Populations

Efficacy and safety analyses will be conducted on intention-to-treat (ITT) and per-protocol (PP)

populations. These are defined as follows:

Intention-to-treat population: **All patients randomized** constitute this population, whether the patients continued to receive antibiotic therapy or not.

Per-protocol population: This group consists of all patients who were randomized, received the duration of antibiotic therapy assigned (within ± 48 hours), had day-30 (primary outcome) follow-up, and for whom no major protocol deviations were documented throughout the study period.

Major protocol deviations are as follow:

- Randomization (in error) despite not fulfilling study entry criteria
- Receipt of antibiotic(s) not considered to follow usual standards of care/ local guidelines
- Receipt of antibiotic(s) prescribed at insufficient doses
- Deviation from the CRP algorithm (>48 hours) for the individual arm
- Difference of duration of antibiotic therapy >48 hours in the fixed arms
- Switch to another arm during trial

3.2 Analyses and methods

3.2.1 Baseline demographics and clinical data

Baseline demographics and clinical characteristics will be described study-wide and by study site. Demographic data will include age, gender and ethnicity. (At the Geneva site, education level and other socio-economic factors will be assessed for the cost-effectiveness analyses.)

Baseline clinical data include, but are not limited to, severity of illness at the time of infection presentation (by qSOFA score), source of bacteremia, infection acquisition type (community-acquired versus nosocomial), baseline microbiologic data (e.g., presence of a multiresistant bacterial infection at inclusion; see definition below), hematologic status (e.g., transient neutropenia before randomization), CRP kinetics (peak and rapidity of decline), and renal function.

Summary tables (descriptive statistics and/or frequency tables) will be provided for baseline demographic and clinical variables as appropriate. Continuous variables will be summarized with descriptive statistics (mean and standard deviation, median and range). Frequency counts and percentage of subjects within each category will be provided for categorical data. Missing data will be noted throughout.

3.2.2 Clinical efficacy analyses

As laid out in the protocol, a non-inferiority hypothesis is postulated, with the expectation that seven days (fixed) of antibiotic therapy and an individualized, CRP-guided duration of therapy will be non-inferior to 14 days of antibiotic therapy in patients meeting entry criteria for this trial. Clinical efficacy analyses will be performed by intervention group on the PP and ITT populations.

We will report the incidence of clinical failure within 30 days of antibiotic therapy start (or post-randomization) (primary outcome time point), and within 60 and 90 days in the three arms. To test the hypothesis of non-inferiority at the pre-specified margin of 10 percentage points, we will perform a generalized linear regression model with a log link and binomial distribution reporting risk differences of clinical failure between each intervention arm (fixed short antibiotic therapy and individualized duration based on CRP arm) compared to the control arm (fixed long antibiotic therapy). The treatment group will be the main predictor with the control arm (“fixed long”) as the

reference and the model will be adjusted for the study centre.[1] We will conclude non-inferiority of the "fixed short" arm compared to the "fixed long" (14 days) if the 95% upper bound of the risk difference in clinical failure between both arms is less than the 10 percentage points' non-inferiority margin. Similarly, we will conclude that the "individualized arm" is non-inferior to the "fixed long" if the 95% upper bound of risk difference is less than the 10 percentage points' non-inferiority margin. These interpretations will be done in one sequence as "treatment group" is defined by three categories. We will also present risk ratios or odds ratios with 95% confidence intervals.

Additionally, provided we have sufficient data (at least 5 events per variable[2]), we will perform the same statistical analyses for any individual primary outcome components, including relapse, local suppurative complication that was not present at infection onset, distal complications of the initial infection, restarting of Gram-negative-directed antibiotic therapy after its initial discontinuation and all-cause mortality within 30 days.

Univariate and multivariate analyses will be conducted to assess associations between clinical failure and the following potential risk factors:

- Age, gender, antibiotic choice (by class), antibiotic route (iv only vs. iv/po sequential therapy), anatomic focus of primary infection, comorbidity status, infection acquisition type (community vs. nosocomial), time to appropriate antibiotic therapy, and severity of illness at the time of diagnosis

For multivariate regression, we will employ a parsimonious model. The following variables will be forced into the model: age, gender, comorbidity status, severity of illness at presentation (by qSOFA); while otherwise, only variables emerging in univariate regression models as significantly associated with clinical failure (e.g., antibiotic choice, infecting organism, etc.) will be included.

Variables emerging from univariate analyses with an arbitrary *p* value cutoff of ≤.20 will be included in the multivariate analyses.

Secondary outcome variables will be compared using the Chi^2 or Fischer exact test for categorical variables and Student's t or Mann-Whitney nonparametric tests for continuous variables. For binary secondary outcomes (clinical failure through days 60 and 90, all-cause mortality, antibiotic-related adverse events, emergence of bacterial resistance in those with recurrence), we will also report risk differences with 95% confidence intervals in the "fixed short" and individualized arms compared to "fixed long" arm using a generalized linear regression model with a log link and binomial distribution. For continuous secondary outcomes (total number of antibiotic days and length of hospital stay), we will report median and interquartile range.

Special considerations: a blinded panel to evaluate patients with clinical failure

While the definition of clinical failure seeks to be as objective as possible, one of its five criteria has an element of subjectivity to it (to wit, "*the restarting of Gram-negative-directed antibiotic therapy after its initial discontinuation due to clinical worsening suspected to be due to the initial infecting organism and for which there is no alternate diagnosis/pathogen suspected*"). For this reason, the cases of all patients deemed to fulfill, at first assessment, the definition of "clinical failure" by day 30 due to the criterion above will be submitted for final evaluation to a panel of study investigators coming from other sites (at least one investigator per site) and blinded to the patients' duration of antibiotic therapy. Investigators will be asked to confirm the assessment of failure or, in the case of disagreement, supply their explanation for any alternate assessment. In cases of discordance among investigators, a majority vote will determine the final call.

3.2.4 Safety and other secondary outcome analyses

Frequency and severity grade of AE considered possibly, probably or definitely related to the study antibiotic will be described by system organ class and by Medical Dictionary for Regulatory Activities (MedDRA) preferred term. Frequency of AEs will be reported and compared among intervention groups with Fisher's exact or the Chi-square test, as appropriate.

3.2.5 Missing data

The absence ("missingness") of data will be noted throughout all reporting, with notation of numerators and denominators for all outcomes. If more than 5% of data on the primary outcome are missing, multiple imputation (MI) methods may be applied. In this model, if one or more observations of "X" is missing, values are simulated from their complete conditional distribution given other X values. For each of the X values (observed and possibly simulated), a MI analysis is conducted. The reported estimates are averaged over all these simulations, and thereby incorporate the error due to "missingness" in a natural and principled manner.
Such may be the primary analysis, but for sensitivity purposes, the following sensitivity analyses may also be conducted:

1) Assume all missing observations in the treatment arms (7 days or individualized duration) and control arm (14 days) had the best possible outcome
2) Assume all missing observations in the treatment and control arms had the worst possible outcome
3) Assume all missing observations in the treatment arms had the best possible outcome and all missing observations in the control arm had the worst possible outcome
4) Assume all missing observations in the treatment arms had the worst possible outcome and all missing observations in the control arm had the best possible outcome

4 Salient definitions

Resistant bacterium. A bacterium with acquired (not intrinsic) resistance to at least one agent in one class of antibacterial agents.

Multidrug-resistant bacterium. In accordance with the European Centre for Disease Prevention and Control's recent proposal for standard definitions of acquired resistance, we define a multidrug-resistant pathogen as one that is resistant to at least one agent in ≥3 classes of antimicrobial agents.[3]

Appropriate empiric antibiotic therapy. All Gram-negative pathogens identified in blood cultures are susceptible to the initial antibiotic(s).

Inappropriate empiric antibiotic therapy. At least one Gram-negative pathogen identified in blood cultures has *in vitro* non-susceptibility to all initial antibiotics or antibiotic combinations.

5 References

1. Food & Drug Administration. Guidance for Industry. E9 Statistical Principles for Clinical Trials. 1998; http://www.fda.gov/downloads/drugs/.../guidances/ucm073137.pdf.
2. Vittinghoff E, McCulloch CE. Relaxing the rule of ten events per variable in logistic and Cox regression. *Am J Epidemiol.* 2007;165(6):710-718.

3. Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect.* 2012;18(3):268-281.