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Multicentre, randomised, open-label, phase IV-III study to evaluate the efficacy of cloxacillin plus fosfomycin versus cloxacillin alone in adult patients with methicillin-susceptible Staphylococcus aureus bacteraemia: study protocol for the SAFO trial.

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Multicentre, randomised, open-label, phase IV-III study to evaluate the efficacy of cloxacillin plus fosfomycin versus cloxacillin alone in adult patients with methicillin-susceptible *Staphylococcus aureus* bacteraemia: study protocol for the SAFO trial.

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Abstract

Introduction:

Methicillin-susceptible *Staphylococcus aureus* (MSSA) bacteraemia is a frequent condition, with high mortality rates. There is a growing interest in identifying new therapeutic regimens able to reduce therapeutic failure and mortality observed with the standard of care of beta-lactam monotherapy. *In vitro* and small-scale studies have found synergy between cloxacillin and fosfomycin against *S. aureus*. Our aim is to test the hypothesis that cloxacillin plus fosfomycin achieves higher treatment success than cloxacillin alone in patients with MSSA bacteraemia.

Methods:

We will perform a superiority, randomised, open-label, phase IV-III, two-armed parallel group (1:1) clinical trial at 20 Spanish tertiary hospitals. The trial will be conducted in accordance with Standard Protocol Items: Recommendation for Interventional Trials (SPIRIT) guidelines. Adults (≥18 years) with isolation of MSSA from at least one blood culture ≤72 hours before inclusion with evidence of infection, will be randomly allocated to receive either cloxacillin 2g/4h intravenous (IV) plus fosfomycin 3 g/6h IV or cloxacillin 2g/4h IV alone for seven days. After the first week, sequential treatment and total duration of antibiotic therapy will be determined according to clinical criteria by the attending physician.

Primary endpoints: 1) Treatment success at day 7, a composite endpoint comprising all the following criteria: patient alive, stable or with improved quick-SOFA score, afebrile and with negative blood cultures for MSSA at day 7. 2) Treatment success at Test of Cure visit (TOC): patient alive and no isolation of MSSA in blood culture or at another sterile site from day 8 until TOC (12 weeks after randomisation).

We assume a rate of treatment success of 74% in the cloxacillin group. Accepting alpha risk of 0.05 and beta risk of 0.2 in a two-sided test, 183 subjects will be required in each of the control

and experimental groups to obtain statistically significant difference of 12% (considered clinically significant).

Ethics and dissemination: Ethical approval has been obtained from the Ethics Committee of Bellvitge University Hospital (AC069/18) and from the Spanish Medicines and Healthcare Product Regulatory Agency (AEMPS, AC069/18), and is valid for all participating centres under existing Spanish legislation. The results will be presented at international meetings and will be made available to patients and funders.

The protocol has been approved by AEMPS with the Trial Registration Number EudraCT 2018-001207-37. ClinicalTrials.gov Identifier: NCT03959345.

Strengths and limitations of this study

- This strategic trial is intended to offer clinicians the best antibiotic treatment for MSSA bacteraemia and to determine whether combining cloxacillin and fosfomycin might improve outcomes compared with cloxacillin alone.
- The primary endpoints are strong composite outcomes that will assess mortality, clinical and microbiological failure at 7 and 90 days after randomisation.
- The multicentre nature of the study supports the generalisability of the results.
- The major limitation will be the open-label design. Nevertheless, the participation of a blinded adjudication committee, which will evaluate the key study endpoints, will mitigate the observer bias inherent in the open-label design.
- Given the increased risk of sodium overload, patients with cardiac failure and hepatic cirrhosis will be excluded.

Introduction

Staphylococcus aureus is one of the most common causes of bacteraemia and endocarditis in industrialised countries, and has particularly high hospitalisation and mortality rates (and associated costs) [1,2]. Healthcare exposure and the increasing use of invasive devices have contributed to the high burden of the disease [3].

Mortality rates due to methicillin-susceptible *Staphylococcus aureus* (MSSA) bacteraemia range between 20 and 30%. Mortality has been linked to factors such as age, co-morbidities, source of infection, pathogen virulence elements and optimisation of antibiotic treatment [4]. Complicated *S. aureus* bacteraemia, defined as the persistence of positive blood cultures during the first week of treatment or haematogenous seeding, is common, and is an indicator of poor prognosis [5]. Indeed, every continued day of bacteraemia has been associated with a higher risk of mortality [6,7].

Even though MSSA bacteraemia is a common and life-threatening infection, the optimum treatment and its duration has not been well established. Nor is it clear whether monotherapy is better than combination therapy. For over 50 years, the standard treatment of MSSA bacteraemia has been cloxacillin monotherapy [8]. Today, there is a growing interest in identifying new therapeutic regimens able to reduce the rate of therapeutic failure and improve the outcomes obtained with the standard of care.

Strategies combining cloxacillin with aminoglycosides have not shown any significant improvement in patients' outcomes, and have been associated with a higher risk of nephrotoxicity [9]. A randomised multicentre study conducted in the UK, which included around 1000 patients and compared the efficacy of the rifampicin combination with the standard treatment for *S. aureus* bacteraemia, did not show a reduction in early or late mortality for the combined therapy compared with monotherapy [10]. Nor did two recent studies comparing a beta-lactam and daptomycin combination with beta-lactams in

monotherapy to treat MSSA bacteraemia show any differences in mortality between groups [11, 12].

Among the combinations that might improve the outcome of patients with MSSA bacteraemia, cloxacillin plus fosfomycin is an appealing strategy. Fosfomycin is a bactericidal antibiotic which inhibits synthesis of N-acetylmuramic acid, a precursor of bacterial wall peptidoglycan, and is highly active against most strains of *S. aureus* [13]. Cross-resistance with other antibiotic groups is very uncommon. Nevertheless, because of the risk of the development of resistance when administered as monotherapy, fosfomycin must be administered in combination with another antibiotic. *In vitro* and small scale studies have demonstrated a synergistic effect of cloxacillin plus fosfomycin against *S. aureus*, and several different beta-lactam combinations have been successfully used in difficult-to-treat *S. aureus* infections [14, 15, 16].

In a recent multicentre trial, we showed that daptomycin plus fosfomycin in methicillin-resistant *S.aureus* (MRSA) bacteraemia achieved better outcomes in younger severely ill patients and faster clearance of bacteraemia than daptomycin alone [17]. To date, however, no randomised studies evaluating the efficacy of cloxacillin plus fosfomycin for treating MSSA bacteraemia have been published or registered in the Clinical Trials database.

Objective: To test the hypothesis that combining cloxacillin plus fosfomycin during the initial seven days of treatment achieves better outcomes than cloxacillin alone in patients with MSSA bacteraemia.

Methods and analysis

Study design and setting

We will perform a multicentre, superiority, randomised, open-label, phase IV-III, two-armed parallel group (1:1) clinical trial. Patients will be recruited from 20 tertiary hospitals in Spain (a list of study sites is available in the Supplementary material). The trial has been registered in the EudraCT and ClinicalTrials databases. The protocol follows the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) initiatives, and the results will be presented in accordance with the Consolidated Standards of Reporting Trials (CONSORT) statement[18] [19].

Study population

Inclusion criteria:

- Subjects aged ≥ 18 years;
- At least one blood culture positive for MSSA ≤72 hours before inclusion, with evidence of active infection;
- Written informed consent from the participant or the legal representative.

Exclusion criteria:

- Severe clinical status with expected death <24h.
- Severe hepatic cirrhosis (Child-Pugh C).
- Moderate-severe cardiac chronic failure (NYHA III-IV).
- Prosthetic endocarditis.
- History of significant allergy to β-lactams or fosfomycin (defined as previous type 1 hypersensitivity reaction to any β-lactams or fosfomycin, or history of serious non-type 1 hypersensitivity reaction to any penicillin or fosfomycin).
- Known *S. aureus* fosfomycin non-susceptibility.

- Polymicrobial bacteraemia with more than one microorganism in blood cultures.
- A positive pregnancy test or pregnancy or lactation at the time of inclusion.
- Myasthenia gravis.
- Participation in another clinical trial.
- Previous participation in the present clinical trial.
- Social problems, cognitive or psychiatric impairment which might be expected to affect adherence to the protocol.
- Acute SARS-CoV2 infection.

Intervention

Patients will be randomly assigned to receive intravenous cloxacillin 2g every four hours plus fosfomycin 3 g every six hours, or to receive cloxacillin 2 g every four hours intravenously for the duration of seven days. If creatinine clearance is <30 mL/min, cloxacillin will be administered at dose of 2g every six hours. The fosfomycin dose will be adjusted according to creatinine clearance, as explained in Table 1.

This treatment will be administered during the first seven days after randomisation. After the first week, the choice of antibiotic strategy and the duration of overall antibiotic treatment will be determined according to clinical criteria by the attending physician, based on current guidelines. Uncomplicated bacteraemia will be treated for 10-14 days, and complicated bacteraemia for 4-6 weeks at least, depending on the source of the infection and other clinical considerations. Removal of a focus of infection as soon as possible and performance of echocardiogram will be prioritised. The assessment schedule is summarised in Table 2. A schematic diagram of study design is shown in Figure 1.

Outcomes

Efficacy will be analysed by intention to treat in all randomised patients, using a hierarchical testing procedure in the following order: treatment success at day 7 followed by treatment success at TOC visit.

Primary endpoints:

Treatment success at day 7 is a composite outcome defined by all the following criteria met after randomisation:

- Patient alive at day 7 AND
- Clinical improvement measured by stable or improved quick SOFA score (compared with baseline) at day 7 AND
- Patient afebrile at day 7 AND
- Negative MSSA blood cultures at day 7.

Treatment success at TOC visit, defined by presence of all of the following:

- Patient alive at TOC;
- No isolation of MSSA in blood culture or at another sterile site from day 8 until the TOC visit (12 weeks after randomisation). In case of patients with a prolonged course of antibiotic treatment (more than 10 weeks), the TOC visit will be performed two weeks after the end of treatment (EOT).

Treatment failure is defined by the presence of one of the following conditions: all-cause mortality at TOC, withdrawal from the study due to adverse events related to the treatment, requirement of an additional MSSA-active antibiotic until day 7, and lack of clinical improvement at day 7.

Secondary endpoints

Clinical:

- All-cause mortality at day 7, EOT and TOC visit.
- Persistent bacteraemia (at least one positive blood culture) at day 3 and persistent bacteraemia at day 7 after randomisation.
- Microbiological relapse, defined by at least one positive blood culture for MSSA at least 72 hours after a preceding negative culture.
- Microbiological treatment failure, defined by a positive sterile site culture for MSSA at least 14 days after randomisation.
- Number of patients with persistent and relapsing bacteraemia.
- Number of patients with complicated bacteraemia, defined as persistent bacteraemia, endocarditis or metastatic emboli, presence of prosthetic devices.
- Length of intensive care unit stay.
- Duration of intravenous antibiotic treatment.

We will perform exploratory subgroup analyses for patients at high risk (those with persistent bacteraemia, metastatic infection, unknown focus of bacteraemia, endocarditis, and pneumonia).

Microbiological:

- *In vitro* cloxacillin plus fosfomycin combination synergy.
- Emergence of fosfomycin-resistant strains during therapy in the combination treatment arm.
- Operon *agr* functionality and its relationship with Minimum Inhibitory Concentration (MIC) changes to vancomycin (VAN) and daptomycin (DAP) and with biofilm production.
- VAN and DAP MIC as markers of complications during bacteraemia.
- Whole genome sequencing and its changes in patients with treatment failure.

Pharmacological:

Patients recruited at the coordinating centre (Bellvitge University Hospital) will be included in a pharmacological sub-study, after obtaining additional signed informed consent. The variables assessed will be:

- Minimum and maximum concentration in steady state of fosfomycin and cloxacillin, and pharmacokinetic variability of these concentrations.
- Associations between pharmacokinetic parameters and efficacy.

Safety:

Safety of cloxacillin plus fosfomycin as compared with cloxacillin alone.

Follow-up and data collection

During the first week of treatment, all patients will be assessed at days 1, 3 and 7 by a member of the investigating team, and followed up daily by an infectious diseases specialist. Scheduled visits are reported in Table 2. A follow-up visit will be arranged for all participants at EOT (48h after the last dose of antibiotic treatment) and at TOC. At this last visit, a structured telephone interview will be performed to assess outcomes.

All data will be recorded on a secure web application used for building and managing online databases (REDCap). Authorised staff will be free to examine the records for quality assurance and audit purposes.

Endpoint assessment

The primary endpoints will be assessed by a committee comprising three independent senior infectious disease specialists with extensive experience in *S. aureus* bacteraemia and endocarditis. This committee will be blinded to treatment allocation and to patient identification. Committee members will receive a data extract containing patients'

demographical data, comorbidities, source of infection, quick SOFA score at baseline and day 7, date and results of blood and sterile cultures between randomisation and TOC, as well as date of death if applicable.

Statistical analysis plan

Sample size

Prior data indicate a success rate in the cloxacillin alone group of 74% [11]. To achieve a success rate in the experimental group of 86% (i.e., an absolute difference of 12%, considered as clinically significant) we will need 183 experimental subjects and 183 control subjects to reject the null hypothesis of an equal success rate with a probability of 80%. The probability of type I error associated with this test is 5%, and a drop-out rate of 5% has been anticipated.

Allocation

Participants will be block randomised to receive monotherapy or combination using an internet-based, concealed computer-generated random allocation sequence. Random blocks will be of size 4 or 6. The randomised sequence allocation will be stored in the Biostatistics Unit at Biomedical Research Institute of Bellvitge (IDIBELL) and will not be available to any member of the research team.

Data analysis

The main analysis will be performed for the intention to treat population, which will include all randomised patients included in the study with a primary outcome assessment. If no statistical significance is detected by day 7 in the hierarchy, then no further hypothesis testing will be performed. The analysis will be repeated in the per protocol population. All patients who receive at least one dose of treatment will be included in the safety analysis.

The Chi-square test will be used to test the binary endpoints of the success rate. The relative risk for success rate will be calculated, accompanied by 95% confidence intervals. The time-to-event outcomes, including the time of response, and overall survival will be estimated using

the Kaplan-Meier method [20]. All analyses and data management will be performed with R software, version 4.0.4 or superior [21].

Monitoring

Monitoring plans

The data monitoring board will ensure the correct progress of the study in terms of safety, and also the sample size assumptions.

Harms - Data Safety and Monitoring Board (DSMB)

An independent DSMB will review safety data and provide advice about the continuation, modification and/or termination of the study, as well as adherence to the protocol, recruitment, outcomes and additional data related to participants' safety. The DSMB will be composed by specialists in pharmacology, biostatistics and infectious diseases. The review by the DSMB will be performed when half of the sample size will be reached.

Adverse events reporting and quantification

An adverse event will be defined as any injury related to medical management occurring during the patient's participation in the study, even if it is not related to the study medication.

An adverse drug event will be defined as any medication-related adverse event occurring during the patient's participation in the clinical trial.

An adverse drug reaction will be defined as any 'adverse drug event' occurring when the medication is used as directed and at the usual dosage.

Serious adverse event or reaction will be defined as an event or reaction that:

- Results in death;
- Is life-threatening;
- Causes persistent or significant disability;
- Causes a congenital anomaly/birth defect;

- Requires in-patient hospitalisation or prolongation of existing hospitalisation (not related to basal diseases).

Adverse drug events of particular interest for the study

- Hypokalaemia and hypocalcaemia: blood analysis will be performed every 2-3 days during the first week to permit potassium and calcium control. Furthermore, administration of potassium supplement will be recommended from the first day of treatment to avoid this complication.
- Sodium overload: since both fosfomycin and cloxacillin carry a high sodium load, daily physical examination and administration of a low dose of a diuretic such as furosemide will be recommended to avoid hypertension, oedema and acute cardiac failure.

Reporting

Any adverse events occurring during the patient's participation in the clinical trial will be recorded on the clinical chart by the principal investigator at each scheduled visit. The principal investigator will record its possible relationship to the study drug.

The electronic case report form should record only the following: serious adverse drug events; adverse events (of any degree) related to the study medication, in the opinion of the PI; adverse events (of any degree) leading to modification of the dosage of the study drug or its interruption/early discontinuation; adverse events of particular interest for the study.

The sponsor will be notified of all serious adverse events within 24 h of their occurrence.

Trial status

The SAFO trial opened its first recruitment site on 31 May 2019. The first patient was enrolled on 1 July 2019. Follow up is expected to be completed by May 2022.

Declaration

Ethics

The trial will be conducted in accordance with the principles of the most recent Declaration of Helsinki (agreed by the 64th World Medical Association General Assembly in 2013), the Good Clinical Practice (GCP) guidelines, and the current legislation. The investigator is responsible for guaranteeing that the clinical trial is conducted in compliance with the directives established by the International Conference on Harmonisation GCP guidelines and with local legislation.

The study was authorised by the Spanish Medicines and Healthcare Products Regulatory Agency (AEMPS, 18-0905) and by the Bellvitge University Hospital Ethics committee (AC069/18).

The principal investigator or collaborator at each site will provide patients with the information sheet, and s/he will explain the nature of the study and the objectives and clarify any doubts. Written informed consent will be obtained from all patients or from their legal representatives (LRs) if they lack capacity, before enrolment. Patients (or their LRs) are free to withdraw from the trial at any time; this will be explicitly stated on the patient's information sheet.

Patients' personal and clinical information will be managed in accordance with European Regulation 2016/679 and Spanish legislation. Patients' data will be anonymised; each patient will be identified by a code. Only the study physician and collaborators will have access to patients' clinical histories. Consequently, patients' identities will not be revealed to any other person, except in cases of medical emergency or if required by law. Access to patient information will be restricted to the study physician and collaborators, the health authorities (AEMPS), the Clinical Research Ethics Committee, and personnel authorised by the sponsor when they need to check the data and procedures used in the study, but always maintaining the confidentiality of the information in accordance with the current legislation. The trial protocol was approved by the research ethics committee on 28 March 2019 and by the AEMPS on 8 April 2019. The informed consent form and information sheet were approved by the research ethics committee on 28 March 2019. The emendation was approved by the research ethics committee and by the AEMPS on 29 November 2019.

Data sharing plan and dissemination

Sharing of data generated by this project is an essential part of our proposed activities and will be carried out in several different ways. We would wish to make our results available both to the community of scientists interested in infectious diseases and the biology of *Staphylococcus aureus* to avoid unintentional duplication of research.

The preliminary results will be presented at international and national infectious diseases conferences and will be published in peer-reviewed journals. The results will also be made available to patients, caregivers and funders through press and social media communications. A corporative *Twitter* account will be created to establish direct contact with the general public and other health-care professionals. Any formal presentation or publication of data collected from this study will be considered as a joint publication by the participating investigators and will follow the recommendations of the International Committee of Medical Journal Editors (ICMJE).

Individual participant data that underlie the results, after deidentification (text, tables, figures, and appendices) will be available immediately following publication and ending 5 years following article publication. Data will be shared with researchers who provide a methodologically sound proposal to achieve aims in the approved suggestions. Propositions should be directed to the corresponding author.

Patients and public involvement

Patients will not be involved in either the enrolment or the execution of the trial, or in the assessment of the interventions. However, before the beginning of the study, a number of patients with previous *S. aureus* bacteraemia were contacted by phone to obtain their feedback about the study.

Protocol amendments

No protocol modifications will become effective until approved by the relevant authorities and by the Drug Research Ethics Committee (CEIm). Exceptions will be made for any changes to protect patients from imminent harm and those concerning exclusively logistic or administrative aspects.

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Authors' contributions

SG, GC, JC and MP conceived and designed the study. SG, GC and MP wrote and revised the manuscript. PH and SB critically reviewed the protocol. All authors have read and approved the final manuscript.

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Competing interests

GH received a research grant from ERN (19PNJ145).

List of abbreviation

MSSA: Methicillin-susceptible *Staphylococcus aureus* bacteraemia. TOC: test of cure. SPIRIT: Standard Protocol Items: Recommendation for Interventional Trials. CONSORT: Consolidated Standards of Reporting Trials. SOFA: Sequential Organ Failure Assessment. MRSA: Methicillin-resistant Staphylococcus aureus. EOT: End of Treatment. GCP: Good Clinical Practice.

Tables

Table 1. Fosfomycin dosage adjusted to renal function.

Creatinine clearance (mL/min)	Fosfomycin dosage
>40	3 g every 6 hours
20-40	3 g every 12 hours
10-20	3g every 24 hours
<10	3 g every 48 hours
Haemodialysis	3 g after haemodialysis
Continuous renal replacement therapy	3 g every 24 hours

Abbreviations: mL: millilitre; min: minute.

Table 2. The SAFO evaluation schedule.

Visit						Unscheduled	
Day	Screening	0	3	7	EOT	Visit[1]	TOC
All patients							
Eligibility criteria	X						
Pregnancy test[2]	X						
Informed consent	X						
Randomisation		X					
Clinical evaluation		X	X	X	X	X	X[3]
Quick SOFA score		X		X			
Blood cultures		X	X	X	X	X	X[3]
Blood count and biochemical analysis[4]		X	X	X		X	X[3]
Adverse events record			X	X	X	X	X
Concomitant medication		X	X	X	X		
Subgroup of patients with PK/PD sub analysis							
Lithium heparin blood sample (2x 5mL)				X			

[1]Unscheduled visit will be performed only in case of clinical infectious symptoms and signs.

[2]Pregnancy test will be performed only in woman of childbearing age.

[3]In absence of infective symptoms, clinical assessment may be made by phone call; blood culture and blood analysis will not be necessary.

[4]Complete blood count, biochemical analysis (C-reactive protein-, creatinine, urea, creatinine clearance, AST, ALT, bilirubin, sodium, potassium, calcium, acid-base analysis) and coagulation test (prothrombin test/INR).

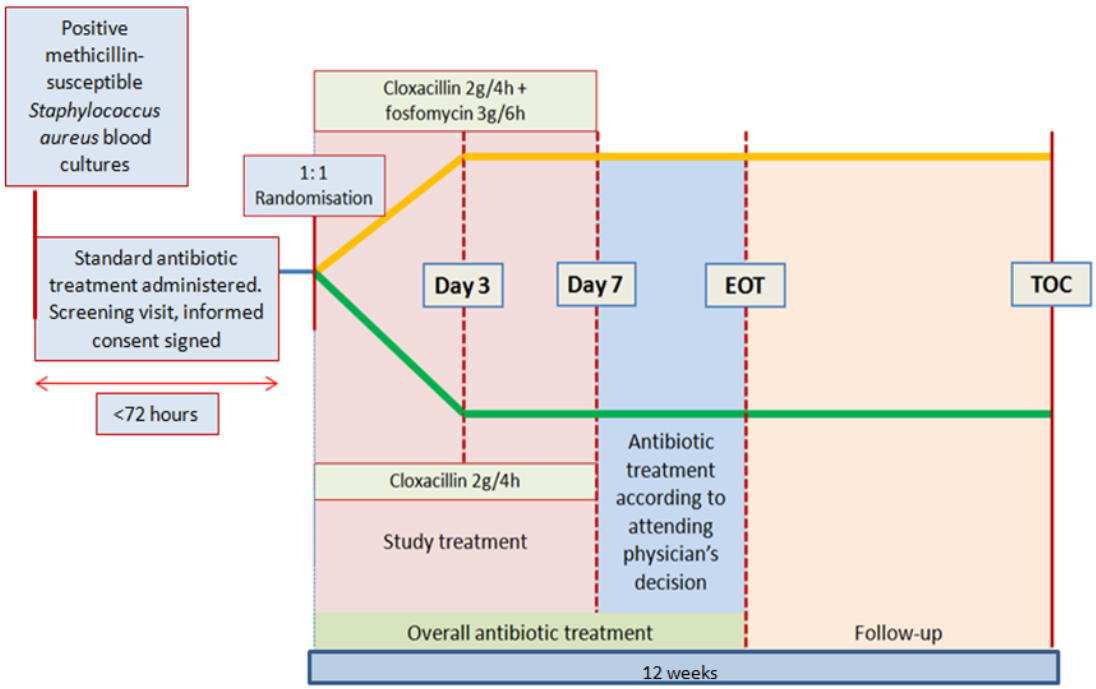
Figures

Figure 1. Study design.

Supplementary material

List of study sites: Hospital Universitari de Bellvitge, Hospitalet de Llobregat, Barcelona; Hospital Universitari Clínic de Barcelona; Hospital Universitari Santa Creu i Sant Pau, Barcelona; Hospital Universitari Parc de Salut Mar, Barcelona; Hospital Universitari Joan XXIII, Tarragona; Hospital Universitari Arnau de Vilanova, Lleida; Hospital Universitari Mútua de Terrassa, Barcelona; Corporació Sanitaria Parc Taulí, Sabadell, Barcelona; Hospital Universitari Sant Joan, Reus; Hospital Universitario M.Broggi, Sant Joan Despí, Barcelona; Hospital Universitario 12 de Octubre, Madrid; Hospital Universitario Virgen Macarena, Seville; Hospital Universitario de Cruces, Barakaldo; Hospital Universitario Lucus Augusti, Lugo; Hospital Clínico Universitario de Zaragoza, Zaragoza; Hospital Universitario Ramón y Cajal, Madrid; Hospital Universitari Germans Trias, Badalona; Hospital Universitario Álvaro Cunqueiro, Vigo; Hospital de Barcelona, Barcelona; Hospital Universitario La Paz, Madrid.

Other hospitals may be added during the course of the clinical trial.



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List of study sites: Hospital Universitari de Bellvitge, Hospitalet de Llobregat, Barcelona; Hospital Universitari Clínic de Barcelona; Hospital Universitari Santa Creu i Sant Pau, Barcelona; Hospital Universitari Parc de Salut Mar, Barcelona; Hospital Universitari Joan XXIII, Tarragona; Hospital Universitari Arnau de Vilanova, Lleida; Hospital Universitari Mútua de Terrassa, Barcelona; Corporació Sanitaria Parc Taulí, Sabadell, Barcelona; Hospital Universitari Sant Joan, Reus; Hospital Universitario M.Broggi, Sant Joan Despí, Barcelona; Hospital Universitario 12 de Octubre, Madrid; Hospital Universitario Virgen Macarena, Seville; Hospital Universitario de Cruces, Barakaldo; Hospital Universitario Lucus Augusti, Lugo; Hospital Clínico Universitario de Zaragoza, Zaragoza; Hospital Universitario Ramón y Cajal, Madrid; Hospital Universitari Germans Trias, Badalona; Hospital Universitario Álvaro Cunqueiro, Vigo; Hospital de Barcelona, Barcelona; Hospital Universitario La Paz, Madrid.

Other hospitals may be added during the course of the clinical trial.

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	5
	2b	All items from the World Health Organization Trial Registration Data Set	
Protocol version	3	Date and version identifier	
Funding	4	Sources and types of financial, material, and other support	21
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1
	5b	Name and contact information for the trial sponsor	21
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	12
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	12
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	6
	6b	Explanation for choice of comparators	6
Objectives	7	Specific objectives or hypotheses	7
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	8

Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	8
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	8
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	10
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	10
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	10
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	23
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	13
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	13
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	13

Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	13
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	13
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	12
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
Methods: Data collection, management, and analysis			
Data collection methods	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	12
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	12
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	13
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	12
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	13
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and 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	14

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

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Multicentre, randomised, open-label, phase IV-III study to evaluate the efficacy of cloxacillin plus fosfomycin versus cloxacillin alone in adult patients with methicillin-susceptible Staphylococcus aureus bacteraemia: study protocol for the SAFO trial.

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Keywords:	INFECTIOUS DISEASES, MICROBIOLOGY, Clinical trials < THERAPEUTICS

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Multicentre, randomised, open-label, phase IV-III study to evaluate the efficacy of cloxacillin plus fosfomycin versus cloxacillin alone in adult patients with methicillin-susceptible *Staphylococcus aureus* bacteraemia: study protocol for the SAFO trial.

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Abstract

Introduction:

Methicillin-susceptible *Staphylococcus aureus* (MSSA) bacteraemia is a frequent condition, with high mortality rates. There is a growing interest in identifying new therapeutic regimens able to reduce therapeutic failure and mortality observed with the standard of care of beta-lactam monotherapy. *In vitro* and small-scale studies have found synergy between cloxacillin and fosfomycin against *S. aureus*. Our aim is to test the hypothesis that cloxacillin plus fosfomycin achieves higher treatment success than cloxacillin alone in patients with MSSA bacteraemia.

Methods:

We will perform a superiority, randomised, open-label, phase IV-III, two-armed parallel group (1:1) clinical trial at 20 Spanish tertiary hospitals. The trial will be conducted in accordance with Standard Protocol Items: Recommendation for Interventional Trials (SPIRIT) guidelines. Adults (≥18 years) with isolation of MSSA from at least one blood culture ≤72 hours before inclusion with evidence of infection, will be randomly allocated to receive either cloxacillin 2g/4h intravenous (IV) plus fosfomycin 3 g/6h IV or cloxacillin 2g/4h IV alone for seven days. After the first week, sequential treatment and total duration of antibiotic therapy will be determined according to clinical criteria by the attending physician.

Primary endpoints: 1) Treatment success at day 7, a composite endpoint comprising all the following criteria: patient alive, stable or with improved quick-SOFA score, afebrile and with negative blood cultures for MSSA at day 7. 2) Treatment success at Test of Cure visit (TOC): patient alive and no isolation of MSSA in blood culture or at another sterile site from day 8 until TOC (12 weeks after randomisation).

We assume a rate of treatment success of 74% in the cloxacillin group. Accepting alpha risk of 0.05 and beta risk of 0.2 in a two-sided test, 183 subjects will be required in each of the control

and experimental groups to obtain statistically significant difference of 12% (considered clinically significant).

Ethics and dissemination: Ethical approval has been obtained from the Ethics Committee of Bellvitge University Hospital (AC069/18) and from the Spanish Medicines and Healthcare Product Regulatory Agency (AEMPS, AC069/18), and is valid for all participating centres under existing Spanish legislation. The results will be presented at international meetings and will be made available to patients and funders.

The protocol has been approved by AEMPS with the Trial Registration Number EudraCT 2018-001207-37. ClinicalTrials.gov Identifier: NCT03959345.

Strengths and limitations of this study

- This strategic trial is intended to determine whether combining cloxacillin and fosfomycin might improve outcomes compared with cloxacillin alone.
- The primary endpoints are strong composite outcomes that will assess mortality, clinical and microbiological failure at 7 and 90 days after randomisation.

-A blinded adjudication committee will evaluate the key study endpoints and mitigate the observer bias inherent in the open-label design.

- Given the increased risk of sodium overload, patients with cardiac failure and hepatic cirrhosis will be excluded.

Introduction

Staphylococcus aureus is one of the most common causes of bacteraemia and endocarditis in industrialised countries, and has particularly high hospitalisation and mortality rates (and associated costs) [1,2]. Healthcare exposure and the increasing use of invasive devices have contributed to the high burden of the disease [3].

Mortality rates at 90 days due to methicillin-susceptible *Staphylococcus aureus* (MSSA) bacteraemia range between 20 and 30% [4][5]. Mortality has been linked to factors such as age, co-morbidities, source of infection, pathogen virulence elements and optimisation of antibiotic treatment [6]. Complicated *S. aureus* bacteraemia is common, and is an indicator of poor prognosis [7]. Indeed, every continued day of bacteraemia has been associated with a higher risk of mortality [8][9].

Even though MSSA bacteraemia is a common and life-threatening infection, up to date, it is not clear whether combination therapy could reduce duration of bacteremia or reduce mortality compared with the current standard of care (monotherapy beta-lactams). For over 50 years, the standard treatment of MSSA bacteraemia has been antistaphylococcal penicillin monotherapy [10]. Today, there is a growing interest in identifying new therapeutic regimens able to reduce the rate of therapeutic failure and improve the outcomes obtained with the standard of care.

Strategies combining cloxacillin with aminoglycosides have not shown any significant improvement in patients' outcomes, and have been associated with a higher risk of nephrotoxicity [11]. A randomised multicentre study conducted in the UK, which included around 1000 patients and compared the efficacy of the rifampicin combination with the standard treatment for *S. aureus* bacteraemia, did not show a reduction in early or late mortality for the combined therapy compared with monotherapy [12]. Nor did two recent studies comparing a beta-lactam and daptomycin combination with beta-lactams in

monotherapy to treat MSSA bacteraemia show any differences in mortality between groups [13][4].

Among the combinations that might improve the outcome of patients with MSSA bacteraemia, cloxacillin plus fosfomycin is an appealing strategy. Fosfomycin is a bactericidal antibiotic which inhibits synthesis of N-acetylmuramic acid, a precursor of bacterial wall peptidoglycan, and is highly active against most strains of *S. aureus* [14]. Cross-resistance with other antibiotic groups is very uncommon. Nevertheless, because of the risk of the development of resistance when administered as monotherapy, fosfomycin must be administered in combination with another antibiotic [15]. *In vitro* and small scale studies have demonstrated a synergistic effect of cloxacillin plus fosfomycin against *S. aureus* [16], and several different beta-lactam combinations have been successfully used in difficult-to-treat methicillin-resistant *S. aureus* (MRSA) infections [17][18].

In a recent multicentre trial, we showed that daptomycin plus fosfomycin in MRSA bacteraemia achieved better outcomes in a subgroup of younger severely ill patients and faster clearance of bacteraemia than daptomycin alone [19]. To date, however, no other randomised studies evaluating the efficacy of cloxacillin plus fosfomycin for treating MSSA bacteraemia have been published or registered in the Clinical Trials database.

Objective: To test the hypothesis that combining cloxacillin plus fosfomycin during the initial seven days of treatment achieves better outcomes than cloxacillin alone in patients with MSSA bacteraemia.

Methods and analysis

Study design and setting

We will perform a multicentre, superiority, randomised, open-label, phase IV-III, two-armed parallel group (1:1) clinical trial. Patients will be recruited from 20 tertiary hospitals in Spain (a list of study sites is available in the Supplementary material). The trial has been registered in the EudraCT and ClinicalTrials databases. The protocol follows the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) initiatives, and the results will be presented in accordance with the Consolidated Standards of Reporting Trials (CONSORT) statement[20] [21].

Study population

Inclusion criteria:

- Subjects aged ≥ 18 years;
- At least one blood culture positive for MSSA ≤72 hours before inclusion, with evidence of active infection;
- Written informed consent from the participant or the legal representative.

Exclusion criteria:

- Severe clinical status with expected death <24h.
- Severe hepatic cirrhosis (Child-Pugh C).
- Moderate-severe cardiac chronic failure (NYHA III-IV).
- Prosthetic endocarditis.
- History of significant allergy to β-lactams or fosfomycin (defined as previous type 1 hypersensitivity reaction to any β-lactams or fosfomycin, or history of serious non-type 1 hypersensitivity reaction to any penicillin or fosfomycin).
- Known *S. aureus* fosfomycin non-susceptibility.

- Polymicrobial bacteraemia with more than one microorganism in blood cultures.
- A positive pregnancy test or pregnancy or lactation at the time of inclusion.
- Myasthenia gravis.
- Participation in another clinical trial.
- Previous participation in the present clinical trial.
- Social problems, cognitive or psychiatric impairment which might be expected to affect adherence to the protocol.
- Acute SARS-CoV2 infection.

Intervention

Patients will be randomly assigned to receive intravenous cloxacillin 2g every four hours plus fosfomycin 3 g every six hours, or to receive cloxacillin 2 g every four hours intravenously for the duration of seven days. If creatinine clearance is <30 mL/min, cloxacillin will be administered at dose of 2g every six hours. The fosfomycin dose will be adjusted according to creatinine clearance, as explained in Table 1.

This treatment will be administered during the first seven days after randomisation. After the first week, the choice of antibiotic strategy and the duration of overall antibiotic treatment will be determined according to clinical criteria by the attending physician, based on current guidelines. Uncomplicated bacteraemia (no evidence of complicated bacteraemia) will be treated for 10-14 days, and complicated bacteraemia (defined as infection with hematogenous seeding, progression of infection beyond the primary focus, persistent bacteraemia, skin alterations suggestive of acute systemic infection, presence of noncatheter device, haemodialysis) for 4-6 weeks at least, depending on the source of the infection and other clinical considerations [22][23]. Removal of a focus of infection as soon as possible and performance of echocardiogram will be prioritised. The assessment schedule is summarised in Table 2. A schematic diagram of study design is shown in Figure 1.

Outcomes

Efficacy will be analysed by intention to treat in all randomised patients, using a hierarchical testing procedure in the following order: treatment success at day 7 followed by treatment success at TOC visit. Furthermore, a per-protocol analysis will also be performed.

Primary endpoints:

Treatment success at day 7 from randomization is a composite outcome defined by all the following criteria met after randomisation:

- Patient alive at day 7 AND
- Clinical improvement measured by stable or improved quick SOFA score (compared with baseline) at day 7 AND
- Patient afebrile at day 7 AND
- Negative MSSA blood cultures at day 7.

Treatment success at TOC visit, defined by presence of all of the following:

- Patient alive at TOC;
- No isolation of MSSA in blood culture and/or at another sterile site from day 8 until the TOC visit (12 weeks after randomisation). In case of patients with a prolonged course of antibiotic treatment (more than 10 weeks), the TOC visit will be performed two weeks after the end of treatment (EOT).

Treatment failure is defined by the presence of one of the following conditions: all-cause mortality at TOC, withdrawal from the study due to adverse events related to the treatment, requirement of an additional MSSA-active antibiotic until day 7, and lack of clinical improvement at day 7.

Secondary endpoints

Clinical:

- All-cause mortality at day 7, EOT and TOC visit.
- Persistent bacteraemia (at least one positive blood culture) at day 3 and persistent bacteraemia at day 7 after randomisation.
- Microbiological relapse, defined by at least one positive blood culture for MSSA at least 72 hours after a preceding negative culture.
- Microbiological treatment failure, defined by a positive sterile site culture for MSSA at least 14 days after randomisation.
- Number of patients with persistent and relapsing bacteraemia.
- Number of patients with complicated bacteraemia, defined as persistent bacteraemia, endocarditis or metastatic emboli, presence of prosthetic devices.
- Length of intensive care unit stay.
- Duration of intravenous antibiotic treatment.

We will perform exploratory subgroup analyses for patients at high risk (those with metastatic infection, unknown focus of bacteremia, endocarditis, and pneumonia) for both primary outcomes. On participants with persistent bacteremia subgroup analysis will be focused on treatment success at TOC.

Microbiological:

- *In vitro* cloxacillin plus fosfomycin combination synergy (see Supplementary material).
- Emergence of fosfomycin-resistant strains during therapy in the combination treatment arm.
- Operon *agr* functionality and its relationship with Minimum Inhibitory Concentration (MIC) changes to vancomycin (VAN) and daptomycin (DAP) and with biofilm production.

- VAN and DAP MIC as markers of complications during bacteraemia. Isolates with rising vancomycin MICs are associated with thicker cell walls and dysfunctional *agr* profiles, involved in quorum sensing and activation of *S. aureus* toxins and other virulence factors, this leads to more resistant but less virulent strains.
- Whole genome sequencing and its changes in patients with treatment failure.

Pharmacological:

Patients recruited at the coordinating centre (Bellvitge University Hospital) will be included in a pharmacological sub-study, after obtaining additional signed informed consent. The variables assessed will be:

- Minimum and maximum concentration in steady state of fosfomycin and cloxacillin, and pharmacokinetic variability of these concentrations.
- Associations between pharmacokinetic parameters and efficacy.

Safety:

Safety of cloxacillin plus fosfomycin as compared with cloxacillin alone (See Supplementary material).

Follow-up and data collection

During the first week of treatment, all patients will be assessed at days 1, 3 and 7 by a member of the investigating team, and followed up daily by an infectious diseases specialist. Scheduled visits are reported in Table 2. A follow-up visit will be arranged for all participants at EOT (48h after the last dose of antibiotic treatment) and at TOC. At this last visit, a structured telephone interview will be performed to assess outcomes.

All data will be recorded on a secure web application used for building and managing online databases (REDCap). Authorised staff will be free to examine the records for quality assurance and audit purposes.

Endpoint assessment

The primary endpoints will be assessed by a committee comprising three independent senior infectious disease specialists with extensive experience in *S. aureus* bacteraemia and endocarditis. This committee will be blinded to treatment allocation and to patient identification. Committee members will receive a data extract containing patients' demographical data, comorbidities, source of infection, quick SOFA score at baseline and day 7, date and results of blood and sterile cultures between randomisation and TOC, as well as date of death if applicable.

Statistical analysis plan

Sample size

Prior data indicate a success rate in the cloxacillin alone group of 74% [4]. To achieve a success rate in the experimental group of 86% (i.e., an absolute difference of 12%, considered as clinically significant) we will need 183 experimental subjects and 183 control subjects to reject the null hypothesis of an equal success rate with a probability of 80%. The probability of type I error associated with this test is 5%, and a drop-out rate of 5% has been anticipated.

Allocation

Participants will be block randomised to receive monotherapy or combination using an internet-based, concealed computer-generated random allocation sequence. Random blocks will be of size 4 or 6. The randomised sequence allocation will be stored in the Biostatistics Unit at Biomedical Research Institute of Bellvitge (IDIBELL) and will not be available to any member of the research team.

Data analysis

The main analysis will be performed for the intention to treat population, which will include all randomised patients included in the study with a primary outcome assessment. If no statistical significance is detected by day 7 in the hierarchy, then no further hypothesis testing will be performed. The analysis will be repeated in the per protocol population. All patients who receive at least one dose of treatment will be included in the safety analysis.

The Chi-square test will be used to test the binary endpoints of the success rate. The relative risk for success rate will be calculated, accompanied by 95% confidence intervals. Absolute risk difference and 95% confidence interval will also be reported. The time-to-event outcomes, including the time of response, and overall survival will be estimated using the Kaplan-Meier method [24]. To account for competing risks, cause-specific cox regression models will be used, and event cause cumulative incidence functions will be plotted [25]. All analyses and data management will be performed with R software, version 4.0.4 or superior [26].

Monitoring

Monitoring plans

The data monitoring board will ensure the correct progress of the study in terms of safety, and also the sample size assumptions.

Harms - Data Safety and Monitoring Board (DSMB)

An independent DSMB will review safety data and provide advice about the continuation, modification and/or termination of the study, as well as adherence to the protocol, recruitment, outcomes and additional data related to participants' safety. The DSMB will be composed by specialists in pharmacology, biostatistics and infectious diseases. The review by the DSMB will be performed when half of the sample size will be reached.

Adverse events reporting and quantification

An adverse event will be defined as any injury related to medical management occurring during the patient's participation in the study, even if it is not related to the study medication.

An adverse drug event will be defined as any medication-related adverse event occurring during the patient's participation in the clinical trial.

An adverse drug reaction will be defined as any 'adverse drug event' occurring when the medication is used as directed and at the usual dosage.

Serious adverse event or reaction will be defined as an event or reaction that:

- Results in death;
- Is life-threatening;
- Causes persistent or significant disability;
- Causes a congenital anomaly/birth defect;
- Requires in-patient hospitalisation or prolongation of existing hospitalisation (not related to basal diseases).

Adverse drug events of particular interest for the study

- Hypokalaemia and hypocalcaemia: blood analysis will be performed every 2-3 days during the first week to permit potassium and calcium control. Furthermore, administration of potassium supplement will be recommended from the first day of treatment to avoid this complication.
- Sodium overload: since both fosfomycin and cloxacillin carry a high sodium load, daily physical examination and administration of a low dose of a diuretic such as furosemide will be recommended to avoid hypertension, oedema and acute cardiac failure.

Reporting

Any adverse events occurring during the patient's participation in the clinical trial will be recorded on the clinical chart by the principal investigator at each scheduled visit. The principal investigator will record its possible relationship to the study drug.

The electronic case report form should record only the following: serious adverse drug events; adverse events (of any degree) related to the study medication, in the opinion of the PI; adverse events (of any degree) leading to modification of the dosage of the study drug or its interruption/early discontinuation; adverse events of particular interest for the study.

The sponsor will be notified of all serious adverse events within 24 h of their occurrence.

Trial status

The SAFO trial opened its first recruitment site on 31 May 2019. The first patient was enrolled on 1 July 2019. Follow up is expected to be completed by May 2022.

Declaration

Ethics

The trial will be conducted in accordance with the principles of the most recent Declaration of Helsinki (agreed by the 64th World Medical Association General Assembly in 2013), the Good Clinical Practice (GCP) guidelines, and the current local legislation.

The study was authorised by the Spanish Medicines and Healthcare Products Regulatory Agency (AEMPS, 18-0905) and by the Bellvitge University Hospital Ethics committee (AC069/18).

The principal investigator or collaborator at each site will provide patients with the information sheet, and s/he will explain the nature of the study and the objectives and clarify any doubts. Written informed consent will be obtained from all patients or from their legal representatives (LRs) if they lack capacity, before enrolment (Supplementary file). Patients (or their LRs) are free to withdraw from the trial at any time; this will be explicitly stated on the patient's information sheet.

Patients' personal and clinical information will be managed in accordance with European Regulation 2016/679 and Spanish legislation. The trial protocol was approved by the research

ethics committee on 28 March 2019 and by the AEMPS on 8 April 2019. The informed consent form and information sheet were approved by the research ethics committee on 28 March 2019. The emendation regarding "acude SARS-CoV2 infection" as exclusion criteria was approved by the research ethics committee and by the AEMPS on 29 November 2020.

Data sharing plan and dissemination

Sharing of data generated by this project is an essential part of our proposed activities and will be carried out in several different ways. We would wish to make our results available both to the community of scientists interested in infectious diseases and the biology of *Staphylococcus aureus* to avoid unintentional duplication of research.

The preliminary results will be presented at international and national infectious diseases conferences and will be published in peer-reviewed journals. The results will also be made available to patients, caregivers and funders through press and social media communications. A corporative *Twitter* account will be created to establish direct contact with the general public and other health-care professionals. Any formal presentation or publication of data collected from this study will be considered as a joint publication by the participating investigators and will follow the recommendations of the International Committee of Medical Journal Editors (ICMJE).

Individual participant data that underlie the results, after deidentification (text, tables, figures, and appendices) will be available immediately following publication and ending 5 years following article publication. Data will be shared with researchers who provide a methodologically sound proposal to achieve aims in the approved suggestions. Propositions should be directed to the corresponding author.

Patients and public involvement

Patients will not be involved in either the enrolment or the execution of the trial, or in the assessment of the interventions. However, before the beginning of the study, a number of

patients with previous *S. aureus* bacteraemia were contacted by phone to obtain their feedback about the study.

Protocol amendments

No protocol modifications will become effective until approved by the relevant authorities and by the Drug Research Ethics Committee (CEIm). Exceptions will be made for any changes to protect patients from imminent harm and those concerning exclusively logistic or administrative aspects.

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Authors' contributions

SG, GC, JM, JC, DB, MD, AP, SC and MP conceived and designed the study. SG, GC, MP and JC wrote and revised the manuscript. CT and NP designed and wrote statistical analysis plan. PH and SV critically reviewed the protocol. SG, GC, RSJ, JA, LM, SGZ, JLC, OG, AGG, SI, GGP, EC, LBP, IO, AJS, LELC, GE, MA, MJGP, FG, JRP, MLPB, RB, MTPR, YM, MBLY and GH will contribute to the adquisition of data. All authors have read and approved the final manuscript.

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Competing interests

GH received a research grant from ERN (19PNJ145).

List of abbreviation

MSSA: Methicillin-susceptible *Staphylococcus aureus* bacteraemia. TOC: test of cure. SPIRIT: Standard Protocol Items: Recommendation for Interventional Trials. CONSORT: Consolidated Standards of Reporting Trials. SOFA: Sequential Organ Failure Assessment. MRSA: Methicillin-resistant Staphylococcus aureus. EOT: End of Treatment. GCP: Good Clinical Practice.

Tables

Table 1. Fosfomycin dosage adjusted to renal function.

Creatinine clearance (mL/min)	Fosfomycin dosage
>40	3 g every 6 hours
20-40	3 g every 12 hours
10-20	3g every 24 hours
<10	3 g every 48 hours
Haemodialysis	3 g after haemodialysis
Continuous renal replacement therapy	3 g every 24 hours

Abbreviations: mL: millilitre; min: minute.

538 **Table 2.** The SAFO evaluation schedule.

	Visit						Unscheduled	
	Day	Screening	0	3	7	EOT	Visit[1]	TOC
All patients								
Eligibility criteria		X						
Pregnancy test[2]		X						
Informed consent		X						
Randomisation			X					
Clinical evaluation			X	X	X	X	X	X[3]
Quick SOFA score			X		X			
Blood cultures			X	X	X	X	X	X[3]
Blood count and biochemical analysis[4]			X	X	X		X	X[3]
Adverse events record				X	X	X	X	X
Concomitant medication			X	X	X	X		
Subgroup of patients with PK/PD sub analysis								
Lithium heparin blood sample (2x 5mL)					X			

539 [1]Unscheduled visit will be performed only in case of clinical infectious symptoms and signs.

540 [2]Pregnancy test will be performed only in woman of childbearing age.

541 [3]In absence of infective symptoms, clinical assessment may be made by phone call; blood culture and

542 blood analysis will not be necessary.

543 [4]Complete blood count, biochemical analysis (C-reactive protein-, creatinine, urea, creatinine clearance,

544 AST, ALT, bilirubin, sodium, potassium, calcium, acid-base analysis) and coagulation test (prothrombin

545 test/INR).

Figures

Figure 1. Study design.

Supplementary material

List of study sites: Hospital Universitari de Bellvitge, Hospitalet de Llobregat, Barcelona; Hospital Universitari Clínic de Barcelona; Hospital Universitari Santa Creu i Sant Pau, Barcelona; Hospital Universitari Parc de Salut Mar, Barcelona; Hospital Universitari Joan XXIII, Tarragona; Hospital Universitari Arnau de Vilanova, Lleida; Hospital Universitari Mútua de Terrassa, Barcelona; Corporació Sanitaria Parc Taulí, Sabadell, Barcelona; Hospital Universitari Sant Joan, Reus; Hospital Universitario M.Broggi, Sant Joan Despí, Barcelona; Hospital Universitario 12 de Octubre, Madrid; Hospital Universitario Virgen Macarena, Seville; Hospital Universitario de Cruces, Barakaldo; Hospital Universitario Lucus Augusti, Lugo; Hospital Clínico Universitario de Zaragoza, Zaragoza; Hospital Universitario Ramón y Cajal, Madrid; Hospital Universitari Germans Trias, Badalona; Hospital Universitario Álvaro Cunqueiro, Vigo; Hospital de Barcelona, Barcelona; Hospital Universitario La Paz, Madrid.

Other hospitals may be added during the course of the clinical trial.

Microbiological studies

In fosfomycin resistant SASM strains, synergy studies between cloxacilin and fosfomycin will be assessed by E-test and time-kill assays.

For the time-kill assays 0.25, 0.5 and 1 fold MIC for each antibiotic will be tested. Synergy for the combination is defined as >2 log10 CFU/mL decrease in comparison with that by the most active antibiotic of the combination tested, and antagonism is defined as >2 log10 CFU/mL increase.

For the E-test, we calculate the fractional inhibitory concentration index (FICI) with the following formula: FICI = (MICCF/MICC) + (MICBFC/MICF), where MICCF is the MIC of cloxacillin tested in combination with fosfomycin, MICC and MICF are the MIC of cloxacillin and fosfomycin tested alone, MICFC is the MIC of fosfomycin tested in combination with cloxacillin. Synergy is defined as a FICI ≤0.5, indifference as a FICI between >0.5 and 4 and antagonism as a

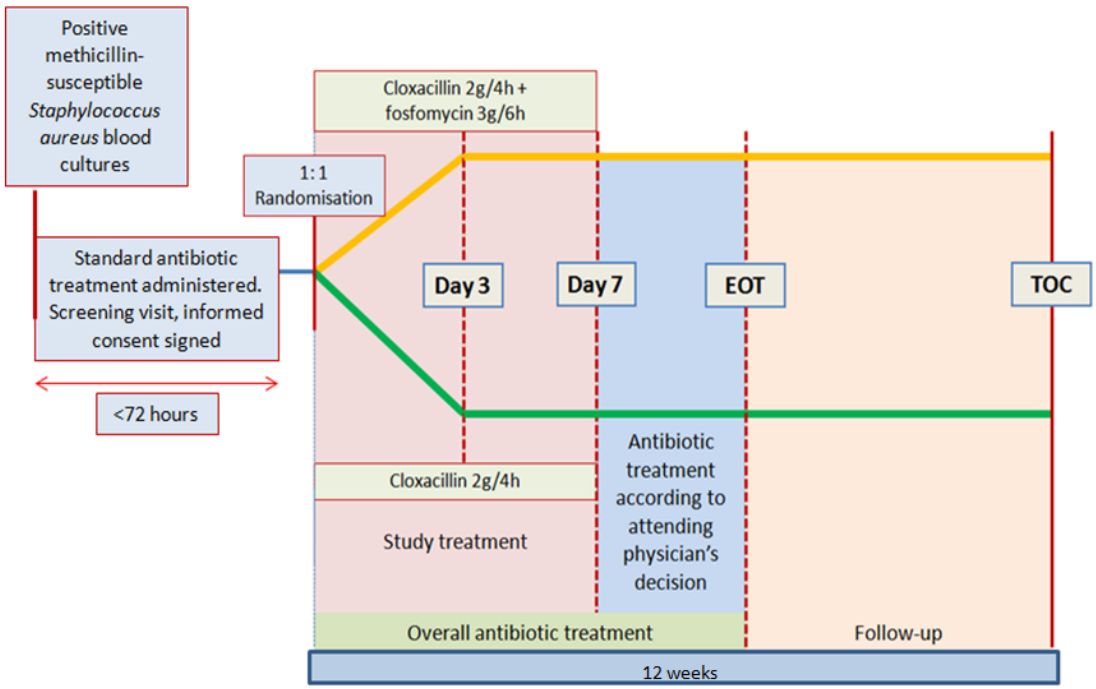
FICI > 4.

Safety outcomes

We will compare incidence of serious adverse events (SAEs) in both group. Particularly, we will assess the incidence of acute cardiac failure, hypokalemia, hypocalcemia, methabolic alcalosis and hypernatremia.

Additional ethical information

Patients' data will be anonymised; each patient will be identified by a code. Only the study physician and collaborators will have access to patients' clinical histories. Consequently, patients' identities will not be revealed to any other person, except in cases of medical emergency or if required by law. Access to patient information will be restricted to the study physician and collaborators, the health authorities (AEMPS), the Clinical Research Ethics Committee, and personnel authorised by the sponsor when they need to check the data and procedures used in the study, but always maintaining the confidentiality of the information in accordance with the current legislation.



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HOJA DE INFORMACIÓN AL PACIENTE

Título del estudio: "Ensayo clínico de fase IV-III, con asignación aleatoria, controlado, abierto y multicéntrico, con dos grupos paralelos, para evaluar la eficacia de la combinación de Cloxacilina y Fosfomicina versus cloxacilina en monoterapia en el tratamiento de la bacteriemia por *Staphylococcus aureus* sensible a la Meticilina"

Código del estudio: HUB-IDIBELL-SAFO-4.3.1.

EudraCT num.: 2018-001207-37

Promotor: Dr. Miquel Pujol i Rojo del Servicio de Enfermedades Infecciosas del Hospital Universitari de Bellvitge

Investigador principal:

Centro:

INTRODUCCIÓN

Nos dirigimos a usted para informarle sobre un estudio de investigación en el que se le invita a participar. El estudio ha sido aprobado por el Comité de Ética de la Investigación con medicamentos y por la Agencia Española de Medicamentos y Productos Sanitarios, de acuerdo a la legislación vigente, el Real Decreto 1090/2015 de 4 de diciembre y el Reglamento Europeo 536/2014 de 16 de abril, por los que se regulan los ensayos clínicos con medicamentos.

Nuestra intención es que usted reciba la información correcta y suficiente para que pueda decidir si acepta o no participar en este estudio. Para ello lea esta hoja informativa con atención y nosotros le aclararemos las dudas que le puedan surgir.

Además, puede consultar con las personas que considere oportuno.

PARTICIPACIÓN VOLUNTARIA

Debe saber que su participación en este estudio es voluntaria y que puede decidir no participar. Si decide participar, puede cambiar su decisión y retirar el consentimiento en cualquier momento, sin que por ello se altere la relación con su médico ni se produzca perjuicio alguno en su atención sanitaria.

OBJETIVO DEL ESTUDIO

La bacteriemia por *Staphylococcus aureus,* que es la enfermedad que usted padece ahora, es una infección muy frecuente y que presenta una elevada mortalidad. El tratamiento actual es la Cloxacilina como único fármaco.

El objetivo es establecer si añadir otro antibiótico (Fosfomicina) al tratamiento habitual con Cloxacilina mejora el pronóstico de esta infección.

HOJA DE INFORMACIÓN AL PACIENTE

DESCRIPCIÓN DEL ESTUDIO

El presente estudio prevé la inclusión de 366 pacientes y se realizará en 15 diferentes hospitales en toda España.

Tanto la Cloxacilina como la Fosfomicina son fármacos que ya están comercializados y se utilizan en la práctica clínica habitual.

El estudio pretende que algunos pacientes además de recibir el tratamiento con cloxacilina (que es el tratamiento habitual de esta infección) reciban también otro antibiótico asociado, la fosfomicina. La combinación de estos dos antibióticos pretende mejorar el control de la infección y así disminuir las complicaciones que esta infección comporta.

Los pacientes recibirán el tratamiento de estudio o el tratamiento habitual. El que Usted reciba el tratamiento de estudio (Cloxacilina + Fosfomicina) o el tratamiento habitual (Cloxacilina sola) será determinado por el azar a través de una asignación por un programa informático. Usted tiene la misma probabilidad que le toque uno u otro tratamiento.

Por el diseño del estudio, tanto su médico habitual como usted sabrán en todo momento que medicación está recibiendo. La medicación será administrada por vía intravenosa.

ACTIVIDADES DEL ESTUDIO

El tratamiento de estudio durará 7 días, después de los cuales el tipo de tratamiento y la duración serán decididos por su médico habitual según la práctica clínica habitual. El seguimiento será de 12 semanas después del comienzo del tratamiento.

Si acepta participar en el estudio, además de las visitas que realizará su equipo médico habitual, se le realizarán 5 visitas extras (el primero, tercero y séptimo día de tratamiento, al final del tratamiento total y después de 12 semanas desde el comienzo del tratamiento). En caso que usted lo necesite (si presentara fiebre por ejemplo) se le realizará una visita extra entre la visita 4 y la visita 5.

Durante las visitas de la 1 a la 5, y en la visita extra si lo requiriese, el equipo investigador realizará una evaluación clínica que incluirá ver si tiene fiebre, conocer su tensión arterial y exploración física general. Comprobará también los resultados de análisis de sangre (hemograma, función renal y hepática, iones y equilibrio ácido-base venoso) que le haya realizado su equipo médico ese día.

También durante todas las visitas se solicitarán 2 muestras de sangre de 10 mL (hemocultivos) para asegurar que la bacteria que le ha producido la enfermedad ha desaparecido de su sangre.

Durante el estudio no se le realizarán analíticas complementarias a las que se realizan en la práctica habitual de su enfermedad.

Las muestras obtenidas se utilizaran para los análisis del estudio, pero no se prevé su almacenamiento una vez concluidos los análisis del estudio.

HOJA DE INFORMACIÓN AL PACIENTE Visita 1	Visita 2	Visita 3	Visita 4	Visita 5
Día de la inclusión	Día +3 desde inicio tratamiento del estudio	Día + 7 desde inicio tratamiento del estudio	A las 48 h de la finalización del ciclo de tratamiento antibiótico completo	+ 12 semanas de la asignación aleatoria
Exploración física	Exploración física	Exploración física	Exploración física	Exploración física
Test de embarazo				
Obtención de muestra de sangre (10 mL)	Obtención de muestra de sangre (10 mL)	Obtención de muestra de sangre (10 mL)	Obtención de muestra de sangre (10 mL)	Obtención de muestra de sangre (10 mL)
En caso de fiebre o síntomas de infección entre la visita 4 y la visita 5, se realizará una visita extraordinaria donde se le realizarás una exploración física y se obtendrán muestras de sangre.				

RIESGOS Y MOLESTIAS DERIVADOS DE SU PARTICIPACIÓN EN EL ESTUDIO

Los fármacos utilizados en este estudio están autorizados para el tratamiento de la enfermedad que Usted padece. Están comercializados desde hace muchos años y se utilizan en la práctica clínica habitual.

Los efectos secundarios principales que pueden presentarse con ambos fármacos son la sobrecarga de sodio y la posibilidad de un descenso del potasio. La sobrecarga de sodio, podría desencadenar episodios de insuficiencia cardíaca y será valorada en las visitas y se administrará tratamiento diurético cuando sea necesario. La hipokalemia (descenso del potasio en sangre), se puede corregir con la administración de potasio a través de sueros o por vía oral. Ambas eventualidades serán valoradas y se realizarán controles y medidas para evitar su desarrollo. Otros efectos secundarios descritos con la fosfomicina son: reacciones cutáneas por hipersensibilidad, aumento transitorio de los enzimas hepáticos, náuseas, diarreas, hipocalcemia (descenso de calcio en sangre) y alcalosis metabólica.

Al ser, tanto la cloxacilina como la fosfomicina, fármacos aprobados por las autoridades sanitarias competentes, existe información al acceso de todo el mundo sobre los efectos secundarios. Por favor, hable con el médico de su estudio para obtener una lista completa de los efectos secundarios comunicados con este fármaco y en cualquier caso se le entregará el prospecto del fármaco.

Si acepta participar al estudio, acepta acudir a las visitas de seguimiento y de notificar cualquier evento adverso que le suceda o cambios en medicación, advirtiendo que, excepto en caso de urgencia, no modifique la medicación que está tomando ni tome otros medicamentos o “plantas medicinales” sin consultar antes con el médico del estudio.

HOJA DE INFORMACIÓN AL PACIENTE

Todos los procedimientos que se realizarán durante el ensayo clínico son procedimientos habituales de la práctica clínica. La participación al ensayo supone la extracción de muestras de sangre (hemocultivos) en diferentes momentos, que no difiere de la práctica clínica habitual. Este procedimiento, aunque de bajo riesgo, en ocasiones puede producir hemorragias, hematomas, molestias, infecciones y/o dolor en el punto de extracción de sangre. También puede sentirse mareado.

Los posibles riesgos derivados del procedimiento realizado para la obtención de estas muestras estarán cubiertos por la póliza del seguro del centro hospitalario.

La realización de pruebas diagnósticas (pruebas de imagen o de obtención de muestras clínicas) o terapéuticas invasivas (drenaje de material purulento, desbridamiento quirúrgico), se realizarán según la práctica clínica habitual. La participación al presente ensayo no supone realizar más pruebas de las necesarias.

POSIBLES BENEFICIOS

El posible beneficio de su participación en el estudio es la mejora del tratamiento de la patología en los futuros pacientes. De todas maneras, es posible que no obtenga ningún beneficio para su salud por participar en este estudio.

ADVERTENCIA RELATIVA AL EMBARAZO

Se realizará un test de embarazo antes del comienzo del estudio en las mujeres en edad fértil. Los fármacos empleados en este estudio deben evitarse durante el embarazo. No hay literatura que haya demostrado toxicidad fetal en caso de uso de Cloxacilina. Para lo que concierne la Fosfomicina, se ha demostrado toxicidad para el feto en animales sólo a dosis que provocarían toxicidad materna.

En caso de producirse un embarazo durante su participación en el estudio debe informar a su médico de inmediato para recibir la asistencia médica adecuada. Se solicitará el consentimiento de la recogida de datos del mismo y de datos de salud del bebé hasta 3 meses después (Ley Orgánica 3/2018 de Protección de Datos Personales y garantía de los derechos digitales).

TRATAMIENTOS ALTERNATIVOS

No existen actualmente tratamientos alternativos que hayan demostrado la superioridad respecto al tratamiento habitual con Cloxacilina. Dada la elevada mortalidad de la enfermedad, en la práctica clínica habitual se utilizan diferentes combinaciones de tratamiento, sin que éstas hayan demostrado ser más eficaces que el tratamiento de la cloxacilina sola. Si el paciente decide no participar en el estudio, podría recibir esta misma combinación u otro tratamiento.

HOJA DE INFORMACIÓN AL PACIENTE

SEGURO

Para la participación en este estudio Usted estará cubierto por la póliza de seguro de cada centro hospitalario. No está previsto contratar un seguro específico.

Le informamos que es posible que su participación en este ensayo clínico pueda modificar las condiciones generales y particulares (cobertura) de sus pólizas de seguros (vida, salud, accidente), por ello, le recomendamos que se ponga en contacto con su compañía de seguros y le informe de su participación en el mismo para determinar si podría afectar a su póliza de seguro actual o en el caso de que vaya a contratar una póliza nueva.

PROTECCIÓN DE DATOS PERSONALES

Tanto el Centro como el Promotor son responsables respectivamente del tratamiento de sus datos y se comprometen al cumplimiento del Reglamento (UE) 2016/679 del Parlamento europeo y del Consejo de 27 de abril de 2016 de Protección de Datos (RGPD), así como al resto de leyes y normativa vigente y aplicable (Ley Orgánica 3/2018 de Protección de Datos Personales y garantía de los derechos digitales).

Los datos recogidos para el estudio estarán identificados mediante un código, de manera que no se incluya información que pueda identificarle (nombre ni apellidos, iniciales, dirección, nº de la seguridad social, etc), sino un código. El código que le identifica será asignado de manera aleatoria a través de un programa informático. Sólo su médico del estudio/colaboradores podrá relacionar dichos datos con usted y con su historia clínica. Por lo tanto, su identidad no será revelada a ninguna otra persona salvo a las autoridades sanitarias, cuando así lo requieran o en casos de urgencia médica.

Las muestras biológicas del estudio también estaran identificadas por su código asignado en el estudio acompañado del código de la visita, cumpliendo con lo expuesto en el párrafo anterior.

Los Comités de Ética de la Investigación, los representantes de la Autoridad Sanitaria en materia de inspección (Agencia Española de Medicamentos y Productos Sanitarios, autoridades sanitarias extranjeras) y el personal autorizado por el Promotor (monitores, auditores), únicamente podrán acceder para comprobar los datos personales, los procedimientos del estudio clínico y el cumplimiento de las normas de buena práctica clínica (siempre manteniendo la confidencialidad de la información).

El tratamiento, la comunicación y la cesión de los datos de carácter personal de todos los participantes se ajustarán a lo dispuesto en esta ley.

De acuerdo a lo que establece la legislación de protección de datos, usted puede ejercer los derechos de acceso, modificación, oposición y cancelación de datos, para lo cual deberá dirigirse a su médico del estudio. Además también puede limitar el tratamiento de datos que sean incorrectos, solicitar una copia o que se trasladen a un tercero (portabilidad) los datos que usted ha facilitado para el estudio. Para ejercitar sus derechos, diríjase al investigador

HOJA DE INFORMACIÓN AL PACIENTE

principal del estudio o al Delegado de protección de datos de la institución, email: dataprotection@idibell.cat.

Si usted decide retirar el consentimiento para participar en este estudio, ningún nuevo dato será añadido a la base de datos, pero los datos que ya se hayan recogido hasta el momento no se podrán eliminar para garantizar la validez de la investigación y cumplir con los deberes legales y los requisitos de autorización de medicamentos. Así mismo tiene derecho a dirigirse a la Agencia de Protección de Datos si no quedara satisfecho.

El Investigador y el Promotor están obligados a conservar los datos recogidos para el estudio al menos hasta 25 años tras su finalización. Posteriormente, su información personal solo se conservará por el centro para el cuidado de su salud y por el promotor para otros fines de investigación científica si usted hubiera otorgado su consentimiento para ello y si así lo permite la ley y requisitos éticos aplicables.

Si se realizara una transferencia de sus datos codificados fuera de la UE a las entidades de nuestro grupo, a prestadores de servicios o a investigadores científicos que colaboren con nosotros, los datos del participante quedarán protegidos con salvaguardas tales como contratos u otros mecanismos por las autoridades de protección de datos. Si el participante quiere saber más al respecto, puede contactar al/ a *la* Delegado de Protección de Datos del promotor [secretariaserveiinfecciosos@bellvitgehospital.cat)

GASTOS Y COMPENSACIÓN

En caso de participar en este estudio, usted no tendrá ningún gasto adicional ocasionado por el estudio. No está prevista ninguna compensación económica por participar en el estudio.

OTRA INFORMACIÓN RELEVANTE

Una descripción de este ensayo clínico estará disponible en http://reec.aemps.es, según exige la legislación española. Cualquier nueva información referente a los fármacos utilizados en el estudio y que pueda afectar a su disposición para participar en el estudio, que se descubra durante su participación, le será comunicada por su médico lo antes posible.

Debe saber que puede ser excluido del estudio si el promotor o los investigadores del estudio lo consideran oportuno, ya sea por motivos de seguridad, por cualquier acontecimiento adverso que se produzca por la medicación en estudio o porque

consideren que no está cumpliendo con los procedimientos establecidos. En cualquiera de los casos, usted recibirá una explicación adecuada del motivo que ha ocasionado su retirada del estudio.

Al firmar la hoja de consentimiento adjunta, acepta cumplir con los procedimientos del estudio que se le han expuesto.

HOJA DE INFORMACIÓN AL PACIENTE

Debe usted saber que es posible que su médico de Atención Primaria tenga conocimiento de su participación en este estudio.

¿QUÉ TRATAMIENTO RECIBIRÉ CUANDO FINALICE EL ENSAYO CLÍNICO?

Cuando acabe su participación recibirá el mejor tratamiento disponible y que su médico considere el más adecuado para su enfermedad, pero es posible que no se le pueda seguir administrando la medicación del estudio. Por lo tanto, ni el investigador ni el promotor adquieren compromiso alguno de mantener dicho tratamiento fuera de este estudio.

CONTACTO EN CASO DE DUDAS

Si durante su participación tiene alguna duda o necesita obtener más información, póngase en contacto con Dr/a _____

y teléfono _____.

CONSENTIMIENTO INFORMADO

Título del estudio: "Ensayo clínico de fase IV-III, con asignación aleatoria, controlado, abierto y multicéntrico, con dos grupos paralelos, para evaluar la eficacia de la combinación de Cloxacilina y Fosfomicina versus Cloxacilina en monoterapia en el tratamiento de la bacteriemia por *Staphylococcus aureus* sensible a la Meticilina"

Código del estudio: HUB-IDIBELL-SAFO-4.3.1.

Promotor: Dr. Miquel Pujol i Rojo del Servicio de Enfermedades Infecciosas del Hospital Universitari de Bellvitge.

Investigador Principal:

Centro:

Yo (nombre y apellidos): _____

- He leído la hoja de información que se me ha entregado.
- He podido hacer preguntas sobre el estudio.
- He recibido suficiente información sobre el estudio.
- He hablado con: _____(**nombre del investigador**)
- Comprendo que mi participación es voluntaria.
- Comprendo que puedo retirarme del estudio cuando quiera, sin tener que dar explicaciones, sin que esto repercuta en mis cuidados médicos.
- Acepto que me comuniquen la información derivada de la investigación que pueda ser relevante para mí salud.

Recibiré una copia firmada y fechada de este documento de consentimiento informado.
Presto libremente mi conformidad para participar en el estudio.

Firma del participante
Fecha: ____/____/____
(Nombre, firma y fecha de puño y letra por el paciente)

Firma del investigador
Fecha: ____/____/____

Cuando se obtenga el CI en personas con capacidad modificada para dar su CI.

Firma del representante legal, familiar o persona vinculada de hecho
Fecha: ____/____/____
(**Nombre, firma y fecha de puño y letra por el representante)**

Firma del investigador
Fecha: ____/____/____

CONSENTIMIENTO INFORMADO ORAL ANTE TESTIGOS

Título del estudio: "Ensayo clínico de fase IV-III, con asignación aleatoria, controlado, abierto y multicéntrico, con dos grupos paralelos, para evaluar la eficacia de la combinación de Cloxacilina y Fosfomicina versus Cloxacilina en monoterapia en el tratamiento de la bacteriemia por *Staphylococcus aureus* sensible a la Meticilina"

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Promotor: Dr. Miquel Pujol i Rojo del Servicio de Enfermedades Infecciosas del Hospital Universitari de Bellvitge.

Investigador Principal:

Centro:

Yo, _____*(nombre y apellidos del testigo*), como testigo, afirmo que en mi presencia se ha informado a D/Dª _____*(nombre y apellidos del participante)* y se ha leído la hoja de información que se le ha entregado sobre el estudio, de modo que:

- Ha podido hacer preguntas sobre el estudio.
- Ha recibido suficiente información sobre el estudio.
- Ha hablado con: _____(nombre del investigador)
- Comprende que su participación es voluntaria.
- Comprende que puede retirarme del estudio cuando quiera, sin tener que dar explicaciones, sin que esto repercuta en sus cuidados médicos.
- Acepta que le comuniquen la información derivada de la investigación que pueda ser relevante para su salud.

Recibiré una copia firmada y fechada de este documento de consentimiento informado.

Firma del testigo
Fecha: ____/____/____
(Nombre, fecha y firma de puño y letra por el paciente/testigo)

Firma del investigador
Fecha: ____/____/____

CONSENTIMIENTO INFORMADO ORAL ANTE TESTIGOS

El participante del estudio ha indicado que no puede leer /escribir.

Un miembro del personal del estudio le ha leído el documento de consentimiento, lo ha revisado y comentado con el participante y se le ha concedido la oportunidad de hacer preguntas o consultarlo con otras personas.

El testigo ha de ser una persona imparcial, ajena al estudio.

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	5
	2b	All items from the World Health Organization Trial Registration Data Set	
Protocol version	3	Date and version identifier	
Funding	4	Sources and types of financial, material, and other support	21
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1
	5b	Name and contact information for the trial sponsor	21
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	12
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	12
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	6
	6b	Explanation for choice of comparators	6
Objectives	7	Specific objectives or hypotheses	7
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	8

Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	8
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	8
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	10
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	10
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	10
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	23
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	13
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	13
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	13

Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	13
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	13
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	12
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
Methods: Data collection, management, and analysis			
Data collection methods	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	12
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	12
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	13
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	12
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	13
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and 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	14

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

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Supplem. file
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Multicentre, randomised, open-label, phase IV-III study to evaluate the efficacy of cloxacillin plus fosfomycin versus cloxacillin alone in adult patients with methicillin-susceptible Staphylococcus aureus bacteraemia: study protocol for the SAFO trial.

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Multicentre, randomised, open-label, phase IV-III study to evaluate the efficacy of cloxacillin plus fosfomycin versus cloxacillin alone in adult patients with methicillin-susceptible *Staphylococcus aureus* bacteraemia: study protocol for the SAFO trial.

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Abstract

Introduction:

Methicillin-susceptible *Staphylococcus aureus* (MSSA) bacteraemia is a frequent condition, with high mortality rates. There is a growing interest in identifying new therapeutic regimens able to reduce therapeutic failure and mortality observed with the standard of care of beta-lactam monotherapy. *In vitro* and small-scale studies have found synergy between cloxacillin and fosfomycin against *S. aureus*. Our aim is to test the hypothesis that cloxacillin plus fosfomycin achieves higher treatment success than cloxacillin alone in patients with MSSA bacteraemia.

Methods:

We will perform a superiority, randomised, open-label, phase IV-III, two-armed parallel group (1:1) clinical trial at 20 Spanish tertiary hospitals. Adults (≥18 years) with isolation of MSSA from at least one blood culture ≤72 hours before inclusion with evidence of infection, will be randomly allocated to receive either cloxacillin 2g/4h intravenous (IV) plus fosfomycin 3 g/6h IV or cloxacillin 2g/4h IV alone for seven days. After the first week, sequential treatment and total duration of antibiotic therapy will be determined according to clinical criteria by the attending physician.

Primary endpoints: 1) Treatment success at day 7, a composite endpoint comprising all the following criteria: patient alive, stable or with improved quick-SOFA score, afebrile and with negative blood cultures for MSSA at day 7. 2) Treatment success at Test of Cure visit (TOC): patient alive and no isolation of MSSA in blood culture or at another sterile site from day 8 until TOC (12 weeks after randomisation).

We assume a rate of treatment success of 74% in the cloxacillin group. Accepting alpha risk of 0.05 and beta risk of 0.2 in a two-sided test, 183 subjects will be required in each of the control and experimental groups to obtain statistically significant difference of 12% (considered clinically significant).

Ethics and dissemination: Ethical approval has been obtained from the Ethics Committee of Bellvitge University Hospital (AC069/18) and from the Spanish Medicines and Healthcare Product Regulatory Agency (AEMPS, AC069/18), and is valid for all participating centres under existing Spanish legislation. The results will be presented at international meetings and will be made available to patients and funders.

The protocol has been approved by AEMPS with the Trial Registration Number EudraCT 2018-001207-37. ClinicalTrials.gov Identifier: NCT03959345.

Strengths and limitations of this study

- The primary endpoints are strong composite outcomes that will assess mortality, clinical and microbiological failure at 7 and 90 days after randomisation.
- The multicentre nature of the study supports the generalisability of the results.
-A blinded adjudication committee will evaluate the key study endpoints and mitigate the observer bias inherent in the open-label design.
- Given the increased risk of sodium overload, patients with cardiac failure and hepatic cirrhosis will be excluded.

Introduction

Staphylococcus aureus is one of the most common causes of bacteraemia and endocarditis in industrialised countries, and has particularly high hospitalisation and mortality rates (and associated costs) [1,2]. Healthcare exposure and the increasing use of invasive devices have contributed to the high burden of the disease [3].

Mortality rates at 90 days due to methicillin-susceptible *Staphylococcus aureus* (MSSA) bacteraemia range between 20 and 30% [4][5]. Mortality has been linked to factors such as age, co-morbidities, source of infection, pathogen virulence elements and optimisation of antibiotic treatment [6]. Complicated *S. aureus* bacteraemia is common, and is an indicator of poor prognosis [7]. Indeed, every continued day of bacteraemia has been associated with a higher risk of mortality [8][9].

Although MSSA bacteraemia is a common and life-threatening infection, it is still unclear whether combination therapy can reduce duration of bacteremia or reduce mortality compared with the current standard of care (monotherapy beta-lactams). For over 50 years, the standard treatment of MSSA bacteraemia has been antistaphylococcal penicillin monotherapy [10]. Today, there is a growing interest in identifying new therapeutic regimens able to reduce the rate of therapeutic failure and improve the outcomes obtained with the standard of care.

Strategies combining cloxacillin with aminoglycosides have not shown any significant improvement in patients' outcomes, and have been associated with a higher risk of nephrotoxicity [11]. A randomised multicentre study conducted in the UK, which included around 1000 patients and compared the efficacy of the rifampicin combination with the standard treatment for *S. aureus* bacteraemia, did not show a reduction in early or late mortality for the combined therapy compared with monotherapy [12]. Nor did two recent studies comparing a beta-lactam and daptomycin combination with beta-lactams in

monotherapy to treat MSSA bacteraemia show any differences in mortality between groups [13][4].

Among the combinations that might improve the outcome of patients with MSSA bacteraemia, cloxacillin plus fosfomycin is an appealing strategy. Fosfomycin is a bactericidal antibiotic which inhibits synthesis of N-acetylmuramic acid, a precursor of bacterial wall peptidoglycan, and is highly active against most strains of *S. aureus* [14]. Cross-resistance with other antibiotic groups is very uncommon. Nevertheless, because of the risk of the development of resistance when administered as monotherapy, fosfomycin must be administered in combination with another antibiotic [15]. *In vitro* and small scale studies have demonstrated a synergistic effect of cloxacillin plus fosfomycin against *S. aureus* [16], and several different beta-lactam combinations have been successfully used in difficult-to-treat methicillin-resistant *S. aureus* (MRSA) infections [17][18].

In a recent multicentre trial, we showed that daptomycin plus fosfomycin in MRSA bacteraemia achieved better outcomes in a subgroup of younger severely ill patients and faster clearance of bacteraemia than daptomycin alone [19]. To date, however, no other randomised studies evaluating the efficacy of cloxacillin plus fosfomycin for treating MSSA bacteraemia have been published or registered in the ClinicalTrials.gov database.

We hypothesize that combining cloxacillin plus fosfomycin during the initial seven days of treatment achieves better outcomes than cloxacillin alone in patients with MSSA bacteraemia. The primary objective of the study is to determine and compare mortality, clinical and microbiological failure at 7 and 90 days after randomisation by allocated treatment.

Methods and analysis

Study design and setting

We will perform a multicentre, superiority, randomised, open-label, phase IV-III, two-armed parallel group (1:1) clinical trial. Patients will be recruited from 20 tertiary hospitals in Spain (a list of study sites is available in the Supplementary material). The trial has been registered in the EudraCT and ClinicalTrials databases. The protocol follows the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) initiatives, and the results will be presented in accordance with the Consolidated Standards of Reporting Trials (CONSORT) statement[20] [21].

Study population

Inclusion criteria:

- Subjects aged ≥ 18 years;
- At least one blood culture positive for MSSA ≤72 hours before inclusion, with evidence of active infection;
- Written informed consent from the participant or the legal representative.

Exclusion criteria:

- Severe clinical status with expected death <24h.
- Severe hepatic cirrhosis (Child-Pugh C).
- Moderate-severe cardiac chronic failure (NYHA III-IV).
- Prosthetic endocarditis.
- History of significant allergy to β-lactams or fosfomycin (defined as previous type 1 hypersensitivity reaction to any β-lactams or fosfomycin, or history of serious non-type 1 hypersensitivity reaction to any penicillin or fosfomycin).
- Known *S. aureus* fosfomycin non-susceptibility.

- Polymicrobial bacteraemia with more than one microorganism in blood cultures.
- A positive pregnancy test or pregnancy or lactation at the time of inclusion.
- Myasthenia gravis.
- Participation in another clinical trial.
- Previous participation in the present clinical trial.
- Social problems, cognitive or psychiatric impairment which might be expected to affect adherence to the protocol.
- Acute SARS-CoV2 infection.

Intervention

Patients will be randomly assigned to receive intravenous cloxacillin 2g every four hours plus fosfomycin 3 g every six hours, or to receive cloxacillin 2 g every four hours intravenously for the duration of seven days. If creatinine clearance is <30 mL/min, cloxacillin will be administered at dose of 2g every six hours. The fosfomycin dose will be adjusted according to creatinine clearance, as explained in Table 1.

This treatment will be administered during the first seven days after randomisation. After the first week, the choice of antibiotic strategy and the duration of overall antibiotic treatment will be determined according to clinical criteria by the attending physician, based on current guidelines. Uncomplicated bacteraemia (no evidence of complicated bacteraemia) will be treated for 10-14 days, and complicated bacteraemia (defined as infection with hematogenous seeding, progression of infection beyond the primary focus, persistent bacteraemia, skin alterations suggestive of acute systemic infection, presence of noncatheter device, haemodialysis) for 4-6 weeks at least, depending on the source of the infection and other clinical considerations [22][23]. Removal of a focus of infection as soon as possible and performance of echocardiogram will be prioritised. The assessment schedule is summarised in Table 2. A schematic diagram of study design is shown in Figure 1.

Outcomes

Efficacy will be analysed by intention to treat in all randomised patients, using a hierarchical testing procedure in the following order: treatment success at day 7 followed by treatment success at TOC visit. Furthermore, a per-protocol analysis will also be performed.

Primary endpoints:

Treatment success at day 7 from randomization is a composite outcome defined by all the following criteria met after randomisation:

- Patient alive at day 7 AND
- Clinical improvement measured by stable or improved quick SOFA score (compared with baseline) at day 7 AND
- Patient afebrile at day 7 AND
- Negative MSSA blood cultures at day 7.

Treatment success at TOC visit, defined by presence of all of the following:

- Patient alive at TOC;
- No isolation of MSSA in blood culture and/or at another sterile site from day 8 until the TOC visit (12 weeks after randomisation). In case of patients with a prolonged course of antibiotic treatment (more than 10 weeks), the TOC visit will be performed two weeks after the end of treatment (EOT).

Treatment failure is defined by the presence of one of the following conditions: all-cause mortality at TOC, withdrawal from the study due to adverse events related to the treatment, requirement of an additional MSSA-active antibiotic until day 7, and lack of clinical improvement at day 7.

Secondary endpoints

Clinical:

- All-cause mortality at day 7, EOT and TOC visit.
- Persistent bacteraemia (at least one positive blood culture) at day 3 and persistent bacteraemia at day 7 after randomisation.
- Microbiological relapse, defined by at least one positive blood culture for MSSA at least 72 hours after a preceding negative culture.
- Microbiological treatment failure, defined by a positive sterile site culture for MSSA at least 14 days after randomisation.
- Number of patients with persistent and relapsing bacteraemia.
- Number of patients with complicated bacteraemia, defined as persistent bacteraemia, endocarditis or metastatic emboli, presence of prosthetic devices.
- Length of intensive care unit stay.
- Duration of intravenous antibiotic treatment.

We will perform exploratory subgroup analyses for patients at high risk (those with metastatic infection, unknown focus of bacteremia, endocarditis, and pneumonia) for both primary outcomes. On participants with persistent bacteremia subgroup analysis will be focused on treatment success at TOC.

Microbiological:

- *In vitro* cloxacillin plus fosfomycin combination synergy (see Supplementary material).
- Emergence of fosfomycin-resistant strains during therapy in the combination treatment arm.
- Operon *agr* functionality and its relationship with Minimum Inhibitory Concentration (MIC) changes to vancomycin (VAN) and daptomycin (DAP) and with biofilm production.

- VAN and DAP MIC as markers of complications during bacteraemia. Isolates with rising vancomycin MICs are associated with thicker cell walls and dysfunctional *agr* profiles. These profiles are involved in quorum sensing, activation of *S. aureus* toxins and other virulence factors, leading to more resistant but less virulent strains.
- Whole genome sequencing and its changes in patients with treatment failure.

Pharmacological:

Patients recruited at the coordinating centre (Bellvitge University Hospital) will be included in a pharmacological sub-study, after obtaining additional signed informed consent. The variables assessed will be:

- Minimum and maximum concentration in steady state of fosfomycin and cloxacillin, and pharmacokinetic variability of these concentrations.
- Associations between pharmacokinetic parameters and efficacy.

Safety:

Safety of cloxacillin plus fosfomycin as compared with cloxacillin alone (See Supplementary material).

Follow-up and data collection

During the first week of treatment, all patients will be assessed at days 1, 3 and 7 by a member of the investigating team, and followed up daily by an infectious diseases specialist. Scheduled visits are reported in Table 2. A follow-up visit will be arranged for all participants at EOT (48h after the last dose of antibiotic treatment) and at TOC. At this last visit, a structured telephone interview will be performed to assess outcomes.

All data will be recorded on a secure web application used for building and managing online databases (REDCap). Authorised staff will be free to examine the records for quality assurance and audit purposes.

Endpoint assessment

The primary endpoints will be assessed by a committee comprising three independent senior infectious disease specialists with extensive experience in *S. aureus* bacteraemia and endocarditis. This committee will be blinded to treatment allocation and to patient identification. Committee members will receive a data extract containing patients' demographical data, comorbidities, source of infection, quick SOFA score at baseline and day 7, date and results of blood and sterile cultures between randomisation and TOC, as well as date of death if applicable.

Statistical analysis plan

Sample size

Prior data indicate a success rate in the cloxacillin alone group of 74% [4]. To achieve a success rate in the experimental group of 86% (i.e., an absolute difference of 12%, considered as clinically significant) we will need 183 experimental subjects and 183 control subjects to reject the null hypothesis of an equal success rate with a probability of 80%. The probability of type I error associated with this test is 5%, and a drop-out rate of 5% has been anticipated.

Allocation

Participants will be block randomised to receive monotherapy or combination using an internet-based, concealed computer-generated random allocation sequence. Random blocks will be of size 4 or 6. The randomised sequence allocation will be stored in the Biostatistics Unit at Biomedical Research Institute of Bellvitge (IDIBELL) and will not be available to any member of the research team.

Data analysis

The main analysis will be performed for the intention to treat population, which will include all randomised patients included in the study with a primary outcome assessment. If no statistical significance is detected by day 7 in the hierarchy, then no further hypothesis testing will be performed. The analysis will be repeated in the per protocol population. All patients who receive at least one dose of treatment will be included in the safety analysis.

The Chi-square test will be used to test the binary endpoints of the success rate. The relative risk for success rate will be calculated, accompanied by 95% confidence intervals. Absolute risk difference and 95% confidence interval will also be reported. The time-to-event outcomes, including the time of response, and overall survival will be estimated using the Kaplan-Meier method [24]. To account for competing risks, cause-specific cox regression models will be used, and event cause cumulative incidence functions will be plotted [25]. All analyses and data management will be performed with R software, version 4.0.4 or superior [26].

Monitoring

Monitoring plans

The data monitoring board will ensure the correct progress of the study in terms of safety, and also the sample size assumptions.

Harms - Data Safety and Monitoring Board (DSMB)

An independent DSMB will review safety data and provide advice about the continuation, modification and/or termination of the study, as well as adherence to the protocol, recruitment, outcomes and additional data related to participants' safety. The DSMB will be composed by specialists in pharmacology, biostatistics and infectious diseases. The review by the DSMB will be performed when half of the sample size will be reached.

Adverse events reporting and quantification

An adverse event will be defined as any injury related to medical management occurring during the patient's participation in the study, even if it is not related to the study medication.

An adverse drug event will be defined as any medication-related adverse event occurring during the patient's participation in the clinical trial.

An adverse drug reaction will be defined as any 'adverse drug event' occurring when the medication is used as directed and at the usual dosage.

Serious adverse event or reaction will be defined as an event or reaction that:

- Results in death;
- Is life-threatening;
- Causes persistent or significant disability;
- Causes a congenital anomaly/birth defect;
- Requires in-patient hospitalisation or prolongation of existing hospitalisation (not related to basal diseases).

Adverse drug events of particular interest for the study

- Hypokalaemia and hypocalcaemia: blood analysis will be performed every 2-3 days during the first week to permit potassium and calcium control. Furthermore, administration of potassium supplement will be recommended from the first day of treatment to avoid this complication.
- Sodium overload: since both fosfomycin and cloxacillin carry a high sodium load, daily physical examination and administration of a low dose of a diuretic such as furosemide will be recommended to avoid hypertension, oedema and acute cardiac failure.

Reporting

Any adverse events occurring during the patient's participation in the clinical trial will be recorded on the clinical chart by the principal investigator at each scheduled visit. The principal investigator will record its possible relationship to the study drug.

The electronic case report form should record only the following: serious adverse drug events; adverse events (of any degree) related to the study medication, in the opinion of the PI; adverse events (of any degree) leading to modification of the dosage of the study drug or its interruption/early discontinuation; adverse events of particular interest for the study.

The sponsor will be notified of all serious adverse events within 24 h of their occurrence.

Trial status

The SAFO trial opened its first recruitment site on 31 May 2019. The first patient was enrolled on 1 July 2019. Follow up is expected to be completed by May 2022.

Declaration

Ethics

The trial will be conducted in accordance with the principles of the most recent Declaration of Helsinki (agreed by the 64th World Medical Association General Assembly in 2013), the Good Clinical Practice (GCP) guidelines, and the current local legislation.

The study was authorised by the Spanish Medicines and Healthcare Products Regulatory Agency (AEMPS, 18-0905) and by the Bellvitge University Hospital Ethics committee (AC069/18).

The principal investigator or collaborator at each site will provide patients with the information sheet, and s/he will explain the nature of the study and the objectives and clarify any doubts. Written informed consent will be obtained from all patients or from their legal representatives (LRs) if they lack capacity, before enrolment (Supplementary file). Patients (or their LRs) are free to withdraw from the trial at any time; this will be explicitly stated on the patient's information sheet.

Patients' personal and clinical information will be managed in accordance with European Regulation 2016/679 and Spanish legislation. The trial protocol was approved by the research

ethics committee on 28 March 2019 and by the AEMPS on 8 April 2019. The informed consent form and information sheet were approved by the research ethics committee on 28 March 2019. The emendation regarding "acude SARS-CoV2 infection" as exclusion criteria was approved by the research ethics committee and by the AEMPS on 29 November 2020.

Data sharing plan and dissemination

Sharing of data generated by this project is an essential part of our proposed activities and will be carried out in several different ways. We would wish to make our results available both to the community of scientists interested in infectious diseases and the biology of *Staphylococcus aureus* to avoid unintentional duplication of research.

The preliminary results will be presented at international and national infectious diseases conferences and will be published in peer-reviewed journals. The results will also be made available to patients, caregivers and funders through press and social media communications. A corporative *Twitter* account will be created to establish direct contact with the general public and other health-care professionals. Any formal presentation or publication of data collected from this study will be considered as a joint publication by the participating investigators and will follow the recommendations of the International Committee of Medical Journal Editors (ICMJE).

Individual participant data that underlie the results, after deidentification (text, tables, figures, and appendices) will be available immediately following publication and ending 5 years following article publication. Data will be shared with researchers who provide a methodologically sound proposal to achieve aims in the approved suggestions. Propositions should be directed to the corresponding author.

Patients and public involvement

Patients will not be involved in either the enrolment or the execution of the trial, or in the assessment of the interventions. However, before the beginning of the study, a number of

patients with previous *S. aureus* bacteraemia were contacted by phone to obtain their feedback about the study.

Protocol amendments

No protocol modifications will become effective until approved by the relevant authorities and by the Drug Research Ethics Committee (CEIm). Exceptions will be made for any changes to protect patients from imminent harm and those concerning exclusively logistic or administrative aspects.

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Authors' contributions

SG, GC, JM, JC, DB, MD, AP, SC and MP conceived and designed the study. SG, GC, MP and JC wrote and revised the manuscript. CT and NP designed and wrote statistical analysis plan. PH and SV critically reviewed the protocol. SG, GC, RSJ, JA, LM, SGZ, JLC, OG, AGG, SI, GGP, EC, LBP, IO, AJS, LELC, GE, MA, MJGP, FG, JRP, MLPB, RB, MTPR, YM, MBLY and GH will contribute to the adquisition of data. All authors have read and approved the final manuscript.

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Competing interests

GH received a research grant from ERN (19PNJ145).

List of abbreviation

MSSA: Methicillin-susceptible *Staphylococcus aureus* bacteraemia. TOC: test of cure. SPIRIT: Standard Protocol Items: Recommendation for Interventional Trials. CONSORT: Consolidated Standards of Reporting Trials. SOFA: Sequential Organ Failure Assessment. MRSA: Methicillin-resistant Staphylococcus aureus. EOT: End of Treatment. GCP: Good Clinical Practice.

Tables

Table 1. Fosfomycin dosage adjusted to renal function.

Creatinine clearance (mL/min)	Fosfomycin dosage
>40	3 g every 6 hours
20-40	3 g every 12 hours
10-20	3g every 24 hours
<10	3 g every 48 hours
Haemodialysis	3 g after haemodialysis
Continuous renal replacement therapy	3 g every 24 hours

Abbreviations: mL: millilitre; min: minute.

Table 2. The SAFO evaluation schedule.

Visit							
Day	Screening	0	3	7	EOT	Unscheduled Visit[1]	TOC
All patients							
Eligibility criteria	X						
Pregnancy test[2]	X						
Informed consent	X						
Randomisation		X					
Clinical evaluation		X	X	X	X	X	X[3]
Quick SOFA score		X		X			
Blood cultures		X	X	X	X	X	X[3]
Blood count and biochemical analysis[4]		X	X	X		X	X[3]
Adverse events record			X	X	X	X	X
Concomitant medication		X	X	X	X		
Subgroup of patients with PK/PD sub analysis							
Lithium heparin blood sample (2x 5mL)				X			

[1]Unscheduled visit will be performed only in case of clinical infectious symptoms and signs.

[2]Pregnancy test will be performed only in woman of childbearing age.

[3]In absence of infective symptoms, clinical assessment may be made by phone call; blood culture and blood analysis will not be necessary.

[4]Complete blood count, biochemical analysis (C-reactive protein-, creatinine, urea, creatinine clearance, AST, ALT, bilirubin, sodium, potassium, calcium, acid-base analysis) and coagulation test (prothrombin test/INR).

Figures

Figure 1. Study design.

Supplementary material

List of study sites: Hospital Universitari de Bellvitge, Hospitalet de Llobregat, Barcelona; Hospital Universitari Clínic de Barcelona; Hospital Universitari Santa Creu i Sant Pau, Barcelona; Hospital Universitari Parc de Salut Mar, Barcelona; Hospital Universitari Joan XXIII, Tarragona; Hospital Universitari Arnau de Vilanova, Lleida; Hospital Universitari Mútua de Terrassa, Barcelona; Corporació Sanitaria Parc Taulí, Sabadell, Barcelona; Hospital Universitari Sant Joan, Reus; Hospital Universitario M.Broggi, Sant Joan Despí, Barcelona; Hospital Universitario 12 de Octubre, Madrid; Hospital Universitario Virgen Macarena, Seville; Hospital Universitario de Cruces, Barakaldo; Hospital Universitario Lucus Augusti, Lugo; Hospital Clínico Universitario de Zaragoza, Zaragoza; Hospital Universitario Ramón y Cajal, Madrid; Hospital Universitari Germans Trias, Badalona; Hospital Universitario Álvaro Cunqueiro, Vigo; Hospital de Barcelona, Barcelona; Hospital Universitario La Paz, Madrid.

Other hospitals may be added during the course of the clinical trial.

Microbiological studies

In fosfomycin resistant SASM strains, synergy studies between cloxacilin and fosfomycin will be assessed by E-test and time-kill assays.

For the time-kill assays 0.25, 0.5 and 1 fold MIC for each antibiotic will be tested. Synergy for the combination is defined as >2 log10 CFU/mL decrease in comparison with that by the most active antibiotic of the combination tested, and antagonism is defined as >2 log10 CFU/mL increase.

For the E-test, we calculate the fractional inhibitory concentration index (FICI) with the following formula: FICI = (MICCF/MICC) + (MICBFC/MICF), where MICCF is the MIC of cloxacillin tested in combination with fosfomycin, MICC and MICF are the MIC of cloxacillin and fosfomycin tested alone, MICFC is the MIC of fosfomycin tested in combination with cloxacillin. Synergy is defined as a FICI ≤0.5, indifference as a FICI between >0.5 and 4 and antagonism as a

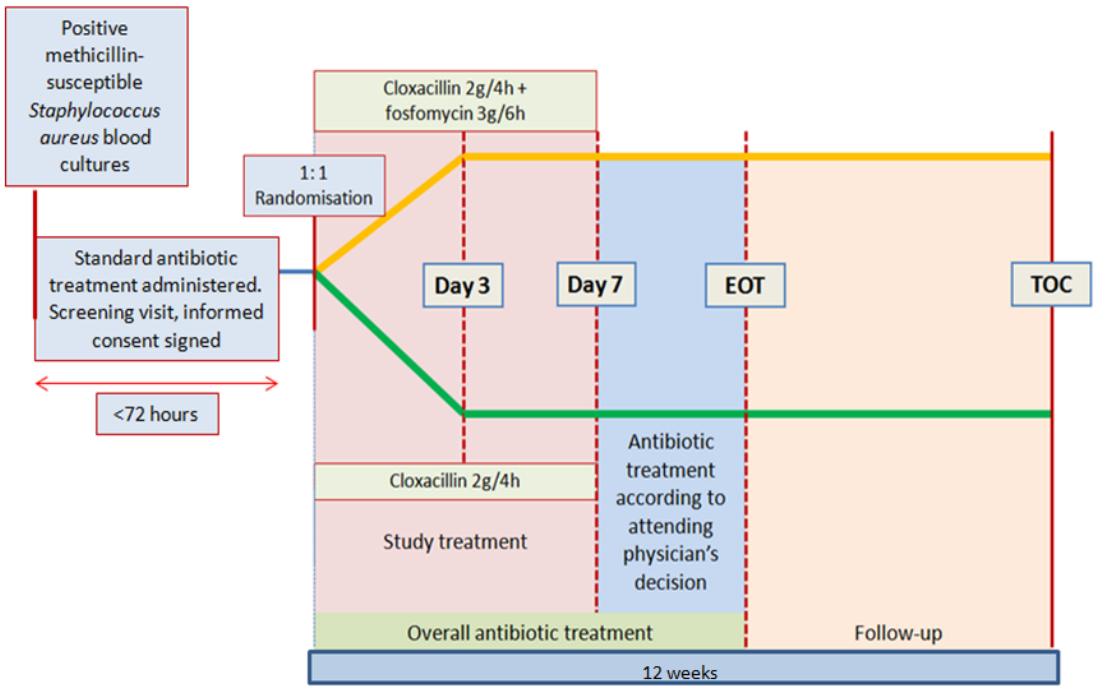
FICI > 4.

Safety outcomes

We will compare incidence of serious adverse events (SAEs) in both group. Particularly, we will assess the incidence of acute cardiac failure, hypokalemia, hypocalcemia, methabolic alcalosis and hypernatremia.

Additional ethical information

Patients' data will be anonymised; each patient will be identified by a code. Only the study physician and collaborators will have access to patients' clinical histories. Consequently, patients' identities will not be revealed to any other person, except in cases of medical emergency or if required by law. Access to patient information will be restricted to the study physician and collaborators, the health authorities (AEMPS), the Clinical Research Ethics Committee, and personnel authorised by the sponsor when they need to check the data and procedures used in the study, but always maintaining the confidentiality of the information in accordance with the current legislation.



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HOJA DE INFORMACIÓN AL PACIENTE

Título del estudio: "Ensayo clínico de fase IV-III, con asignación aleatoria, controlado, abierto y multicéntrico, con dos grupos paralelos, para evaluar la eficacia de la combinación de Cloxacilina y Fosfomicina versus cloxacilina en monoterapia en el tratamiento de la bacteriemia por *Staphylococcus aureus* sensible a la Meticilina"

Código del estudio: HUB-IDIBELL-SAFO-4.3.1.

EudraCT num.: 2018-001207-37

Promotor: Dr. Miquel Pujol i Rojo del Servicio de Enfermedades Infecciosas del Hospital Universitari de Bellvitge

Investigador principal:

Centro:

INTRODUCCIÓN

Nos dirigimos a usted para informarle sobre un estudio de investigación en el que se le invita a participar. El estudio ha sido aprobado por el Comité de Ética de la Investigación con medicamentos y por la Agencia Española de Medicamentos y Productos Sanitarios, de acuerdo a la legislación vigente, el Real Decreto 1090/2015 de 4 de diciembre y el Reglamento Europeo 536/2014 de 16 de abril, por los que se regulan los ensayos clínicos con medicamentos.

Nuestra intención es que usted reciba la información correcta y suficiente para que pueda decidir si acepta o no participar en este estudio. Para ello lea esta hoja informativa con atención y nosotros le aclararemos las dudas que le puedan surgir.

Además, puede consultar con las personas que considere oportuno.

PARTICIPACIÓN VOLUNTARIA

Debe saber que su participación en este estudio es voluntaria y que puede decidir no participar. Si decide participar, puede cambiar su decisión y retirar el consentimiento en cualquier momento, sin que por ello se altere la relación con su médico ni se produzca perjuicio alguno en su atención sanitaria.

OBJETIVO DEL ESTUDIO

La bacteriemia por *Staphylococcus aureus,* que es la enfermedad que usted padece ahora, es una infección muy frecuente y que presenta una elevada mortalidad. El tratamiento actual es la Cloxacilina como único fármaco.

El objetivo es establecer si añadir otro antibiótico (Fosfomicina) al tratamiento habitual con Cloxacilina mejora el pronóstico de esta infección.

HOJA DE INFORMACIÓN AL PACIENTE

DESCRIPCIÓN DEL ESTUDIO

El presente estudio prevé la inclusión de 366 pacientes y se realizará en 15 diferentes hospitales en toda España.

Tanto la Cloxacilina como la Fosfomicina son fármacos que ya están comercializados y se utilizan en la práctica clínica habitual.

El estudio pretende que algunos pacientes además de recibir el tratamiento con cloxacilina (que es el tratamiento habitual de esta infección) reciban también otro antibiótico asociado, la fosfomicina. La combinación de estos dos antibióticos pretende mejorar el control de la infección y así disminuir las complicaciones que esta infección comporta.

Los pacientes recibirán el tratamiento de estudio o el tratamiento habitual. El que Usted reciba el tratamiento de estudio (Cloxacilina + Fosfomicina) o el tratamiento habitual (Cloxacilina sola) será determinado por el azar a través de una asignación por un programa informático. Usted tiene la misma probabilidad que le toque uno u otro tratamiento.

Por el diseño del estudio, tanto su médico habitual como usted sabrán en todo momento que medicación está recibiendo. La medicación será administrada por vía intravenosa.

ACTIVIDADES DEL ESTUDIO

El tratamiento de estudio durará 7 días, después de los cuales el tipo de tratamiento y la duración serán decididos por su médico habitual según la práctica clínica habitual. El seguimiento será de 12 semanas después del comienzo del tratamiento.

Si acepta participar en el estudio, además de las visitas que realizará su equipo médico habitual, se le realizarán 5 visitas extras (el primero, tercero y séptimo día de tratamiento, al final del tratamiento total y después de 12 semanas desde el comienzo del tratamiento). En caso que usted lo necesite (si presentara fiebre por ejemplo) se le realizará una visita extra entre la visita 4 y la visita 5.

Durante las visitas de la 1 a la 5, y en la visita extra si lo requiriese, el equipo investigador realizará una evaluación clínica que incluirá ver si tiene fiebre, conocer su tensión arterial y exploración física general. Comprobará también los resultados de análisis de sangre (hemograma, función renal y hepática, iones y equilibrio ácido-base venoso) que le haya realizado su equipo médico ese día.

También durante todas las visitas se solicitarán 2 muestras de sangre de 10 mL (hemocultivos) para asegurar que la bacteria que le ha producido la enfermedad ha desaparecido de su sangre.

Durante el estudio no se le realizarán analíticas complementarias a las que se realizan en la práctica habitual de su enfermedad.

Las muestras obtenidas se utilizaran para los análisis del estudio, pero no se prevé su almacenamiento una vez concluidos los análisis del estudio.

HOJA DE INFORMACIÓN AL PACIENTE Visita 1	Visita 2	Visita 3	Visita 4	Visita 5
Día de la inclusión	Día +3 desde inicio tratamiento del estudio	Día + 7 desde inicio tratamiento del estudio	A las 48 h de la finalización del ciclo de tratamiento antibiótico completo	+ 12 semanas de la asignación aleatoria
Exploración física	Exploración física	Exploración física	Exploración física	Exploración física
Test de embarazo				
Obtención de muestra de sangre (10 mL)	Obtención de muestra de sangre (10 mL)	Obtención de muestra de sangre (10 mL)	Obtención de muestra de sangre (10 mL)	Obtención de muestra de sangre (10 mL)
En caso de fiebre o síntomas de infección entre la visita 4 y la visita 5, se realizará una visita extraordinaria donde se le realizarás una exploración física y se obtendrán muestras de sangre.				

RIESGOS Y MOLESTIAS DERIVADOS DE SU PARTICIPACIÓN EN EL ESTUDIO

Los fármacos utilizados en este estudio están autorizados para el tratamiento de la enfermedad que Usted padece. Están comercializados desde hace muchos años y se utilizan en la práctica clínica habitual.

Los efectos secundarios principales que pueden presentarse con ambos fármacos son la sobrecarga de sodio y la posibilidad de un descenso del potasio. La sobrecarga de sodio, podría desencadenar episodios de insuficiencia cardíaca y será valorada en las visitas y se administrará tratamiento diurético cuando sea necesario. La hipokalemia (descenso del potasio en sangre), se puede corregir con la administración de potasio a través de sueros o por vía oral. Ambas eventualidades serán valoradas y se realizarán controles y medidas para evitar su desarrollo. Otros efectos secundarios descritos con la fosfomicina son: reacciones cutáneas por hipersensibilidad, aumento transitorio de los enzimas hepáticos, náuseas, diarreas, hipocalcemia (descenso de calcio en sangre) y alcalosis metabólica.

Al ser, tanto la cloxacilina como la fosfomicina, fármacos aprobados por las autoridades sanitarias competentes, existe información al acceso de todo el mundo sobre los efectos secundarios. Por favor, hable con el médico de su estudio para obtener una lista completa de los efectos secundarios comunicados con este fármaco y en cualquier caso se le entregará el prospecto del fármaco.

Si acepta participar al estudio, acepta acudir a las visitas de seguimiento y de notificar cualquier evento adverso que le suceda o cambios en medicación, advirtiendo que, excepto en caso de urgencia, no modifique la medicación que está tomando ni tome otros medicamentos o “plantas medicinales” sin consultar antes con el médico del estudio.

HOJA DE INFORMACIÓN AL PACIENTE

Todos los procedimientos que se realizarán durante el ensayo clínico son procedimientos habituales de la práctica clínica. La participación al ensayo supone la extracción de muestras de sangre (hemocultivos) en diferentes momentos, que no difiere de la práctica clínica habitual. Este procedimiento, aunque de bajo riesgo, en ocasiones puede producir hemorragias, hematomas, molestias, infecciones y/o dolor en el punto de extracción de sangre. También puede sentirse mareado.

Los posibles riesgos derivados del procedimiento realizado para la obtención de estas muestras estarán cubiertos por la póliza del seguro del centro hospitalario.

La realización de pruebas diagnósticas (pruebas de imagen o de obtención de muestras clínicas) o terapéuticas invasivas (drenaje de material purulento, desbridamiento quirúrgico), se realizarán según la práctica clínica habitual. La participación al presente ensayo no supone realizar más pruebas de las necesarias.

POSIBLES BENEFICIOS

El posible beneficio de su participación en el estudio es la mejora del tratamiento de la patología en los futuros pacientes. De todas maneras, es posible que no obtenga ningún beneficio para su salud por participar en este estudio.

ADVERTENCIA RELATIVA AL EMBARAZO

Se realizará un test de embarazo antes del comienzo del estudio en las mujeres en edad fértil. Los fármacos empleados en este estudio deben evitarse durante el embarazo. No hay literatura que haya demostrado toxicidad fetal en caso de uso de Cloxacilina. Para lo que concierne la Fosfomicina, se ha demostrado toxicidad para el feto en animales sólo a dosis que provocarían toxicidad materna.

En caso de producirse un embarazo durante su participación en el estudio debe informar a su médico de inmediato para recibir la asistencia médica adecuada. Se solicitará el consentimiento de la recogida de datos del mismo y de datos de salud del bebé hasta 3 meses después (Ley Orgánica 3/2018 de Protección de Datos Personales y garantía de los derechos digitales).

TRATAMIENTOS ALTERNATIVOS

No existen actualmente tratamientos alternativos que hayan demostrado la superioridad respecto al tratamiento habitual con Cloxacilina. Dada la elevada mortalidad de la enfermedad, en la práctica clínica habitual se utilizan diferentes combinaciones de tratamiento, sin que éstas hayan demostrado ser más eficaces que el tratamiento de la cloxacilina sola. Si el paciente decide no participar en el estudio, podría recibir esta misma combinación u otro tratamiento.

HOJA DE INFORMACIÓN AL PACIENTE

SEGURO

Para la participación en este estudio Usted estará cubierto por la póliza de seguro de cada centro hospitalario. No está previsto contratar un seguro específico.

Le informamos que es posible que su participación en este ensayo clínico pueda modificar las condiciones generales y particulares (cobertura) de sus pólizas de seguros (vida, salud, accidente), por ello, le recomendamos que se ponga en contacto con su compañía de seguros y le informe de su participación en el mismo para determinar si podría afectar a su póliza de seguro actual o en el caso de que vaya a contratar una póliza nueva.

PROTECCIÓN DE DATOS PERSONALES

Tanto el Centro como el Promotor son responsables respectivamente del tratamiento de sus datos y se comprometen al cumplimiento del Reglamento (UE) 2016/679 del Parlamento europeo y del Consejo de 27 de abril de 2016 de Protección de Datos (RGPD), así como al resto de leyes y normativa vigente y aplicable (Ley Orgánica 3/2018 de Protección de Datos Personales y garantía de los derechos digitales).

Los datos recogidos para el estudio estarán identificados mediante un código, de manera que no se incluya información que pueda identificarle (nombre ni apellidos, iniciales, dirección, nº de la seguridad social, etc), sino un código. El código que le identifica será asignado de manera aleatoria a través de un programa informático. Sólo su médico del estudio/colaboradores podrá relacionar dichos datos con usted y con su historia clínica. Por lo tanto, su identidad no será revelada a ninguna otra persona salvo a las autoridades sanitarias, cuando así lo requieran o en casos de urgencia médica.

Las muestras biológicas del estudio también estaran identificadas por su código asignado en el estudio acompañado del código de la visita, cumpliendo con lo expuesto en el párrafo anterior.

Los Comités de Ética de la Investigación, los representantes de la Autoridad Sanitaria en materia de inspección (Agencia Española de Medicamentos y Productos Sanitarios, autoridades sanitarias extranjeras) y el personal autorizado por el Promotor (monitores, auditores), únicamente podrán acceder para comprobar los datos personales, los procedimientos del estudio clínico y el cumplimiento de las normas de buena práctica clínica (siempre manteniendo la confidencialidad de la información).

El tratamiento, la comunicación y la cesión de los datos de carácter personal de todos los participantes se ajustarán a lo dispuesto en esta ley.

De acuerdo a lo que establece la legislación de protección de datos, usted puede ejercer los derechos de acceso, modificación, oposición y cancelación de datos, para lo cual deberá dirigirse a su médico del estudio. Además también puede limitar el tratamiento de datos que sean incorrectos, solicitar una copia o que se trasladen a un tercero (portabilidad) los datos que usted ha facilitado para el estudio. Para ejercitar sus derechos, diríjase al investigador

HOJA DE INFORMACIÓN AL PACIENTE

principal del estudio o al Delegado de protección de datos de la institución, email: dataprotection@idibell.cat.

Si usted decide retirar el consentimiento para participar en este estudio, ningún nuevo dato será añadido a la base de datos, pero los datos que ya se hayan recogido hasta el momento no se podrán eliminar para garantizar la validez de la investigación y cumplir con los deberes legales y los requisitos de autorización de medicamentos. Así mismo tiene derecho a dirigirse a la Agencia de Protección de Datos si no quedara satisfecho.

El Investigador y el Promotor están obligados a conservar los datos recogidos para el estudio al menos hasta 25 años tras su finalización. Posteriormente, su información personal solo se conservará por el centro para el cuidado de su salud y por el promotor para otros fines de investigación científica si usted hubiera otorgado su consentimiento para ello y si así lo permite la ley y requisitos éticos aplicables.

Si se realizara una transferencia de sus datos codificados fuera de la UE a las entidades de nuestro grupo, a prestadores de servicios o a investigadores científicos que colaboren con nosotros, los datos del participante quedarán protegidos con salvaguardas tales como contratos u otros mecanismos por las autoridades de protección de datos. Si el participante quiere saber más al respecto, puede contactar al/ a *la* Delegado de Protección de Datos del promotor [secretariaserveiinfecciosos@bellvitgehospital.cat)

GASTOS Y COMPENSACIÓN

En caso de participar en este estudio, usted no tendrá ningún gasto adicional ocasionado por el estudio. No está prevista ninguna compensación económica por participar en el estudio.

OTRA INFORMACIÓN RELEVANTE

Una descripción de este ensayo clínico estará disponible en http://reec.aemps.es, según exige la legislación española. Cualquier nueva información referente a los fármacos utilizados en el estudio y que pueda afectar a su disposición para participar en el estudio, que se descubra durante su participación, le será comunicada por su médico lo antes posible.

Debe saber que puede ser excluido del estudio si el promotor o los investigadores del estudio lo consideran oportuno, ya sea por motivos de seguridad, por cualquier acontecimiento adverso que se produzca por la medicación en estudio o porque

consideren que no está cumpliendo con los procedimientos establecidos. En cualquiera de los casos, usted recibirá una explicación adecuada del motivo que ha ocasionado su retirada del estudio.

Al firmar la hoja de consentimiento adjunta, acepta cumplir con los procedimientos del estudio que se le han expuesto.

HOJA DE INFORMACIÓN AL PACIENTE

Debe usted saber que es posible que su médico de Atención Primaria tenga conocimiento de su participación en este estudio.

¿QUÉ TRATAMIENTO RECIBIRÉ CUANDO FINALICE EL ENSAYO CLÍNICO?

Cuando acabe su participación recibirá el mejor tratamiento disponible y que su médico considere el más adecuado para su enfermedad, pero es posible que no se le pueda seguir administrando la medicación del estudio. Por lo tanto, ni el investigador ni el promotor adquieren compromiso alguno de mantener dicho tratamiento fuera de este estudio.

CONTACTO EN CASO DE DUDAS

Si durante su participación tiene alguna duda o necesita obtener más información, póngase en contacto con Dr/a _____

y teléfono _____.

CONSENTIMIENTO INFORMADO

Título del estudio: "Ensayo clínico de fase IV-III, con asignación aleatoria, controlado, abierto y multicéntrico, con dos grupos paralelos, para evaluar la eficacia de la combinación de Cloxacilina y Fosfomicina versus Cloxacilina en monoterapia en el tratamiento de la bacteriemia por *Staphylococcus aureus* sensible a la Meticilina"

Código del estudio: HUB-IDIBELL-SAFO-4.3.1.

Promotor: Dr. Miquel Pujol i Rojo del Servicio de Enfermedades Infecciosas del Hospital Universitari de Bellvitge.

Investigador Principal:

Centro:

Yo (nombre y apellidos): _____

- He leído la hoja de información que se me ha entregado.
- He podido hacer preguntas sobre el estudio.
- He recibido suficiente información sobre el estudio.
- He hablado con: _____(**nombre del investigador**)
- Comprendo que mi participación es voluntaria.
- Comprendo que puedo retirarme del estudio cuando quiera, sin tener que dar explicaciones, sin que esto repercuta en mis cuidados médicos.
- Acepto que me comuniquen la información derivada de la investigación que pueda ser relevante para mí salud.

Recibiré una copia firmada y fechada de este documento de consentimiento informado.
Presto libremente mi conformidad para participar en el estudio.

Firma del participante
Fecha: ____/____/____
(Nombre, firma y fecha de puño y letra por el paciente)

Firma del investigador
Fecha: ____/____/____

Cuando se obtenga el CI en personas con capacidad modificada para dar su CI.

Firma del representante legal, familiar o persona vinculada de hecho
Fecha: ____/____/____
(**Nombre, firma y fecha de puño y letra por el representante)**

Firma del investigador
Fecha: ____/____/____

CONSENTIMIENTO INFORMADO ORAL ANTE TESTIGOS

Título del estudio: "Ensayo clínico de fase IV-III, con asignación aleatoria, controlado, abierto y multicéntrico, con dos grupos paralelos, para evaluar la eficacia de la combinación de Cloxacilina y Fosfomicina versus Cloxacilina en monoterapia en el tratamiento de la bacteriemia por *Staphylococcus aureus* sensible a la Meticilina"

Código del estudio: HUB-IDIBELL-SAFO-4.3.1.

Promotor: Dr. Miquel Pujol i Rojo del Servicio de Enfermedades Infecciosas del Hospital Universitari de Bellvitge.

Investigador Principal:

Centro:

Yo, _____*(nombre y apellidos del testigo*), como testigo, afirmo que en mi presencia se ha informado a D/Dª _____*(nombre y apellidos del participante)* y se ha leído la hoja de información que se le ha entregado sobre el estudio, de modo que:

- Ha podido hacer preguntas sobre el estudio.
- Ha recibido suficiente información sobre el estudio.
- Ha hablado con: _____(nombre del investigador)
- Comprende que su participación es voluntaria.
- Comprende que puede retirarme del estudio cuando quiera, sin tener que dar explicaciones, sin que esto repercuta en sus cuidados médicos.
- Acepta que le comuniquen la información derivada de la investigación que pueda ser relevante para su salud.

Recibiré una copia firmada y fechada de este documento de consentimiento informado.

Firma del testigo
Fecha: ____/____/____
(Nombre, fecha y firma de puño y letra por el paciente/testigo)

Firma del investigador
Fecha: ____/____/____

CONSENTIMIENTO INFORMADO ORAL ANTE TESTIGOS

El participante del estudio ha indicado que no puede leer /escribir.

Un miembro del personal del estudio le ha leído el documento de consentimiento, lo ha revisado y comentado con el participante y se le ha concedido la oportunidad de hacer preguntas o consultarlo con otras personas.

El testigo ha de ser una persona imparcial, ajena al estudio.

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	5
	2b	All items from the World Health Organization Trial Registration Data Set	
Protocol version	3	Date and version identifier	
Funding	4	Sources and types of financial, material, and other support	21
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1
	5b	Name and contact information for the trial sponsor	21
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	12
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	12
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	6
	6b	Explanation for choice of comparators	6
Objectives	7	Specific objectives or hypotheses	7
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	8

Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	8
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	8
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	10
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	10
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	10
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	23
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	13
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	13
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	13

Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	13
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	13
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	12
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
Methods: Data collection, management, and analysis			
Data collection methods	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	12
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	12
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	13
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	12
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	13
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and 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	14

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

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Supplem. file
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.