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Effect of the oXiris® membrane on microcirculation after cardiac surgery under cardiopulmonary by-pass: study protocol for a randomized controlled trial (OXICARD Study).

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Strengths and limitations of this study

- We hypothesized that early (during CPB) cytokines adsorption with oXiris® membrane for patients at high risk of inflammatory syndrome following cardiac surgery may improve microcirculation, endothelial function and outcomes.
- This is a prospective, monocentric and randomized trial.
- The trial will be conducted in a tertiary hospital center and will include 70 patients scheduled for cardiac surgery.
- The inclusion criterion is patients with more than 18 years old undergoing elective cardiac surgery under CPB with an expected CPB time > 90 minutes (double valve replacement or valve replacement plus coronary arterial bypass graft (CABG)).
- The primary endpoint will be the microcirculatory flow index (MFI) measured by sublingual microcirculation device at day 1 following cardiac surgery.

Abstract

Introduction

Cytokine storm and endotoxin release during cardiac surgery with cardiopulmonary bypass (CPB) have been related to vasoplegic shock and organ dysfunction. We hypothesized that early (during CPB) cytokines adsorption with oXiris® membrane for patients at high risk of inflammatory syndrome following cardiac surgery may improve microcirculation, endothelial function and outcomes.

Methods and analysis: The Oxicard trial is a prospective, monocentric trial, randomizing 70 patients scheduled for cardiac surgery. The inclusion criterion is patients with more than 18 years old undergoing elective cardiac surgery under CPB with an expected CPB time > 90 minutes (double valve replacement or valve replacement plus coronary arterial bypass graft (CABG)). Patients will be allocated to the intervention group (n=35) or the control group (n=35). In the intervention group, oXiris® membrane will be used on the Prismaflex device (Baxter) at blood pump flow of 450 ml min^{-1} during cardiac surgery under CPB. In the control group, cardiac surgery under CPB will be conducted as usual without oXiris® membrane. An intention-to-treat analysis will be performed. The primary endpoint will be the microcirculatory flow index (MFI) measured by sublingual microcirculation device at day 1 following cardiac surgery. The secondary endpoints will be other microcirculation parameters at CPB end, 6 hours after CPB, at day 1 and at day 2. We also aim to evaluate the occurrence of major cardiovascular and cerebral events (MACE) (e.g., myocardial infarction, stroke, ischemic mesenteric, rescussited cardiac arrest, acute kidney injury) within the first 30 days. Cumulative catecholamine use, intensive care unit (ICU) length of stay, endothelium glycocalyx shedding parameters (syndecan-1, heparan-sulfate and hyaluronic acid), inflammatory cytokines (TNF alpha, IL1 beta, IL 10, IL 6, LPS, endothelin), and endothelial permeability biomarkers (angiopoietin 1, angiopoietin 2, Tie2 soluble receptor and VEGF) will also be evaluated.

Ethics and dissemination: Ethical approval has been obtained from the institutional review board (IRB) of the University Hospital of Amiens (Registration number ID RDB: 2019-A02437-50 in February 2020). Results of the study will be disseminated via peer-reviewed publications and presentations at national and international conferences.

Trial registration: NCT04201119. Registered on 17th of December 2019.

https://www.clinicaltrials.gov/ct2/show/NCT04201119?term=abou+arab&draw=2&rank=3

Background

A "sepsis-like syndrome" is often observed in postoperative cardiac surgery under cardiopulmonary-bypass (CPB), especially when CPB is over 90 minutes (double valve replacement or valve replacement plus coronary arterial bypass graft (CABG)). This condition is due to microcirculation disturbances induced by endothelial dysfunction. Indeed, extracorporeal circulation, associated with aortic clamping induces changes in the blood circulation with a so-called continuous circulation (unlike the usual pulsed circulation), leading to a heterogeneity of organ perfusion. The associated vasoplegic syndrome (SV) in post-operative cardiac surgery is due to acute circulatory failure, defined by persistent low blood pressure, requiring treatment with vasopressors [1] [2] [3]. From a pathophysiological point of view, SV is the result of a complex inflammatory cascade whose origin is multifactorial. This pro-inflammatory state activates complement, platelets and leukocytes which release vasoactive substances. Following CPB, impaired microcirculation is still common and can lead to true organ failure by decreasing oxygen delivery to tissues. Impaired microcirculation after bypass surgery has been well demonstrated with a decrease in microcirculatory flow to the lateral margin of the tongue. The use of sidestream or incident dark field/orthogonal polarized microscopy remains the reference method to clinically assess microcirculation [4][5]. The most easily accessible site at the patient's bedside is the microcirculation of the sublingual mucosa [6] [7] [8]. This pro-inflammatory state can be modulated with the removal of cytokines. The oXiris® (Baxter,IL,USA) membrane is an AN-69 membrane, surface-treated with polyethylenimine (PEI) and grafted with heparin. This property allows the adsorption of inflammatory cytokines and lipopolysaccharide (LPS) [9] [10]. The accepted indication is sepsis or septic shock requiring renal replacement therapy [11][12]. In cardiac surgery, there are no sufficient clinical data to recommend the use of the oXiris® membrane to reduce postoperative inflammatory syndrome. Some studies have demonstrated the feasibility of using the oXiris® membrane during cardiac surgery with CPB with effective removal of inflammatory cytokines [11][12]. However, to date, no studies have established the beneficial effect of cytokine removal on microcirculatory parameters.

We hypothesized that the early use (during CPB) of cytokine adsorption with the oXiris® membrane in patients at high risk of inflammatory syndrome following cardiac surgery may improve microcirculation and hence, clinical outcomes.

Methods

Ethics and study design

The institutional review board (IRB) of the University Hospital of Amiens (Comité de Protection des Personnes Nord-Ouest III, 80054 Amiens, France) approved the study (Registration number ID RDB: 2019-A02437-50 in February 2020). The Oxicard study was conducted in accordance with the Declaration of Helsinki and French law on clinical research [13] and was registered on the 17th of December 2019 on the ClinicalTrials.gov website with the trial identification number NCT04201119. Oxicard trial follows CONSORT Statement [14]. - CONSORT diagram is given in Figure 1. Written informed consent will be obtained from all participants or next of kin.

Study population

The inclusion criteria are as follows:

- Patients with more than 18 years old
- Elective cardiac surgery under CPB with an expected CPB time > 90 minutes (double valve replacement or valve replacement plus coronary arterial bypass graft (CABG))
- Written informed consent from patient or next of kin.

The non-inclusion criteria are as follows:

- Missing informed consent.
- Patient under 18 years old.
- Planned hypothermia <32°C during CPB.
- Emergency surgery
- Acute infective endocarditis
- Immunosuppressive treatment or steroids (prednisone > 0.5mg/kg/day or equivalent)
- AIDS with a CD4 count of <200/mm^3
- Autoimmune disorder

- Transplant recipient
- Advances chronic Kidney Disease (CKD 4 or 5)
- Renal replacement therapy (RRT) in the last 90 days
- Known allergy to study device
- History of confirmed or suspected heparin-induced thrombocytopenia.
- Inclusion in other study within the last 30 days.
- Pregnancy
- Coexisting illness with a high probability of death (inferior to 6 months)

Study protocol

Patients will be randomized into two parallel open-label group. Randomization will be carried out using Clinsight® software, implemented by a data manager. The result of randomization will be displayed as "standard group" or "intervention group». (Figure 1). In the intervention group, cardiac surgery will be conducted with the use of the oXiris® membrane connected to the CPB device.

The oXiris® membrane will be fitted to a dedicated Prismaflex® machine (Baxter,IL,USA) at the maximum flow rate recommended by the pharmaceutical laboratory at a blood flow rate of 450 ml.min^{-1}(Figure 2). The inflow of the Prismaflex® (and the Oxiris membrane) will be positioned between the venous line of CPB and the reservoir, and the outflow between the venous reservoir and the head of the CPB pump. In the standard group, cardiac surgery with CPB without the use of the oXiris® membrane. The conditions for performing CPB are those usually used for this type of surgery in the Anesthesia Department of the Amiens University Hospital, and do not differ from those that would be used outside the scope of this study.

For both arms, monitoring of sublingual microcirculation, primary and second endpoints will be assessed as defined in the data collection section.

There is no provision for ancillary and post-trial care.

Standard procedures

Patients will undergo cardiac surgery as usual and usual postoperative care will be provided.

CPB circuit will be primed (1000 ml crystalloid and 500 ml colloid solution together with 5000 IE heparin) in accordance with institutional guidelines. CPB will be performed by using non-pulsatile flow at 2.5 l · min−1 · m−2, a non-heparin-coated circuit, and a membrane oxygenator (QuadroxTM, Maquet, Hirrlingen, Germany, or Capiox, Terumo, Eschborn, Germany). Blood transfusion will be performed in accordance with the Society of Thoracic Surgeons/ Society of Cardiovascular Anesthesiologists (STS-SCA) transfusion guidelines and administration of coagulation factors will be based on blood samples[15]. Blood samples and all variables will be equally collected and registered in the control group with no modifications from CPB standard practice. The conduct of CPB will be standardized for the study with normothermia, hematocrit more than 20%, mean arterial pressure over 50 mmHg using vasopressor if required.

Standard of care will be given in the ICU after cardiac surgery to all study subjects. Especially for mechanical ventilation, nutrition, sedation, anticoagulation, and blood glucose control, therapy is based on local treatment protocols. In ICU, treatment with vasopressors, inotropes, and fluids is guided by hemodynamic monitoring using echocardiography to achieve a mean arterial pressure of 65 mmHg, a cardiac index over 2.5 ml min-1 m-2 and a diuresis> 0.5 ml kg-1 h-1. All patient will have a continuous cardiac output monitoring with a transpulmonary thermodilution device (Picco, Drager®). Hemodynamics time points assessment will be performed at ICU admission, 6 hours after , at day 1 and at day 2 after surgery.

Outcome measures

The endpoints and definitions are presented in Table 1.

The primary endpoint will be the microcirculatory flow using the MFI (microcirculatory flow index) measured by sublingual microcirculation device at day 1 following cardiac surgery.

Sublingual microcirculation will be visualized and measured using an incident dark field imaging device (Cytocam®, Breadius®, Amsterdam, The Netherlands) derived from the orthogonal polarized spectral (OPS) imaging. Five sequences of 20 seconds each from different adjacent sublingual areas will be recorded. Video sequences will be analyzed off-line by a single rater blinded to the time point of recording, to the study protocol, the surgical procedure, and the clinical status of the patient.

MFI will be assessed manually after dividing the screen in 4 quadrants. According to the classification of Boerma and colleagues [4], MFI values will be notified as follow:

0= no flow, 1= intermittent flow, 2 =sluggish flow, 3 =continuous flow. MFI value is the average value of the four quadrants.

The secondary endpoints will be other microcirculatory flow parameters: the perfused vessel density (PVD), the proportion of perfused vessels (PPV), the total vessel density and the De Backer score recorded by the sublingual microcirculation device (Cytocam®, Breadius®, Amsterdam, The Netherlands) and automatically calculated via a dedicated software (CytoCamtools® software (Braedius Medical, Huizen, the Netherlands) at different time points: prior to CPB, at the end of CPB, 6 hours after CPB, at day 1 and at day 2.

We also aim to evaluate the occurrence within the first 30 days of major cardiovascular and cerebral events (MACE) defined as the occurrence of at least one outcome among the following: successfully resuscitated cardiac arrest, stroke, acute kidney injury, myocardial infarction, mesenteric ischemia. Others outcomes will be in-hospital mortality and at 1-month, cumulative catecholamine use, intensive care unit stay (ICU) (days), hospital stay (days), SOFA score and SAPS II. Several biomarkers will be measured on blood sample. Endothelium glycocalyx shedding markers (syndecan-1, heparan-sulfate and hyaluronic acid), inflammatory cytokines (TNF alpha, IL1 beta, IL 10, IL 6, LPS), and endothelial permeability biomarkers (angiopoietin 1, angiopoietin 2, Tie2 soluble receptor, endothelin-1 and VEGF) will be also evaluated.

Data collection and outcome definitions

The following data will be collected: age (years), gender, body mass index (kg m^{-2}), medical history (coronary disease, peripheral vascular disease, stroke, smoking, diabetes, dyslipidemia, chronic obstructive pulmonary disease, logistic EuroSCORE[16], hypertension, chronic kidney disease, usual medication, surgery type (valve replacement, coronary bypass graft or combined surgery), preoperative left ventricular ejection fraction, baseline hemoglobin, duration of CPB and aortic clamp, intraoperative transfusion, and cumulative dose of vasoactive drugs (dobutamine, norepinephrine, ephedrine, phenylephrine).

Before and after CPB until day 2, the following data will be collected: systolic arterial pressure, diastolic arterial pressure, mean arterial pressure, heart rate, arterial blood

gas parameters (pH, PaO_2, $PaCO_2$, lactate, HCO_3), $ScVO_2$ (%), SOFA score, SAPS II score, vasoactive drug dose, and biological parameters (creatinine, ASAT, ALAT, TP, TCA, platelet, Cardiac troponin, myoglobin). ICU and hospital stays (days) will be recorded. Endothelium glycocalyx shedding (syndecan-1, heparan-sulfate and hyaluronic acid), inflammatory cytokine dosage (TNF alpha, IL1 beta, IL 10, IL 6, LPS, endothelin), and endothelial permeability biomarkers (angiopoietin 1, angiopoietin 2, Tie2 soluble receptor and VEGF) will be collected before CPB, at the end of CPB, 6 h after ending CPB, then at day 1 and day 2 after CPB.

Endpoints will be assessed after cardiac surgery. Adverse events will be declared and notified in the eCRFs.

Standard definitions of postoperative outcomes established by the European Society of Anesthesia were used [17]. Cardiac arrest is defined as the cessation of cardiac mechanical activity, as confirmed by the absence of circulation signs. Stroke isdefined as an embolic, thrombotic, or hemorrhagic cerebral event with persistent residual motor, sensory, or cognitive dysfunction (e.g. hemiplegia, hemiparesis, aphasia, sensory deficit, impaired memory) diagnosed on a cerebral scanner. Acute kidney injury is defined according to Kidney Disease Improving Global Outcomes (KDIGO) criteria as an increase in serum creatinine of over 27 µmol/L within 48 h or diuresis lower than 0.5 mL/kg/h [18]. Myocardial injury is diagnosed by the characteristics presentation, serial changes on 12-lead electrocardiographic suggesting infarction, and arise in cardiac troponin, with at least one value above the 99th percentile of the upper reference limit [19]. Mesenteric ischemia will be confirmed by imaging or exploratory laparotomy and ischemic colitis will be confirmed by gastrointestinal endoscopy or exploratory laparotomy.

Intention-to-treat analysis

Patients with serious adverse events will be analyzed according to their assigned group following the intention-to-treat principle.

Statistical method and Sample size calculation

We predict a baseline mean MFI of 2.8 with a standard deviation (SD) of 0.5. We calculated a sample of 70 patients to show an MFI difference of 0.4 at D1 after surgery in a bilateral test with a type 1 error of 0.05 and a power of 90%. 35 patients in each group (standard and interventional groups)

Primary end point will be compared by a Student test or Wilcoxon-Mann-Whitney test as appropriate. Secondary end points will be assessed using an ANOVA test for repeated measures. MACE and mortality rate will be compared with a chi-2 square test. ICU and hospital stay will be compared using a Student test. Cumulative event curves will be estimated with the Kaplan-Meier procedure (censored at 30 days). Variables between usual and intervention groups will be compared with a Student test, a Wilcoxon-Mann-Whitney test, a chi-2 square or a Fischer exact test as appropriate. A p value of 0.05 will be considered as significant. No intermediate analysis is planned in the trial.

Data management and monitoring

Registration

Data will be collected and registered using electronic case report forms (eCRFs) by a dedicated local research technician. A research coordinator will centralize and verify the data.

Record keeping

Consent forms and eCRFs will be retained for 15 years at the University Hospital of Amiens in accordance with French law.

Study organization

The study promotion is performed by the University Hospital of Amiens, France.

Duration and timeline

Patients from Amiens university hospital can be included during a 2-year period beginning from July 2020.

The processes of developing the protocol, obtaining approval from the ethical committee, obtaining financial support, and developing the eCRFs occurred in 2019. The database should be closed after all participants have been included, followed by data analysis, manuscript writing and submission for publication.

Dissemination

Authors will be involved in disseminating research findings (through attending conferences and co-authoring results 'papers).

Patients or public involvement

Patients or the public will not be involved in the design, or conduct, or reporting, or dissemination plans of our research. Written informed consent will be obtained from all participants or next of kin.

Discussion

The hypothesis is that early use (during CPB) of cytokine adsorption with the oXiris® membrane in patients at high risk of VS following cardiac surgery may improve microcirculation and endothelial function.

Postoperative period following cardiac surgery is associated with an inflammatory state leading to side effects such as vasoplegia and major adverse events[20]. Clinical and experimental data support a link between inflammation with cytokine production and endothelial cell injury [21]. Microcirculatory impairment have also been observed after cardiac surgery [8][22] [23].

Despite normal macrocirculatory parameters, microcirculatory dysfunction resulting in functional oxygen shunting can lead to impairment in oxygen delivery and thus, to organ dysfunction and mortality [24].

Several studies have investigated cytokine adsorption in vitro. Moreover, it has been shown that oXiris® membrane reduces the concentration of cytokines after CPB and during sepsis [9][10]. However, to date, no studies have been performed on the effect of cytokine adsorption on endothelial function and microcirculation.

We hypothesize that systemic microcirculation, assessed by sublingual microcirculation, may be improved when an adsorbent membrane is used to reduce the amount of circulating cytokines.

A pilot study to evaluate the cytokine adsorption effect is an important step before carrying out a larger prospective randomized study with clinical endpoints.

Nevertheless, we will also assess major cardiovascular and cerebral events (stroke, acute kidney injury and myocardial infarction …) in order to evaluate the impact of improved microcirculation on organ dysfunction.

In the event of a positive effect, a multicenter randomized trial will be conducted using morbidity and mortality as primary endpoints.

Trial status

The trial is not yet recruiting.

List of abbreviations

AIDS: Acquired-immune-deficiency-syndrome
CABG: coronary arterial bypass graft
CPB: Cardiopulmonary bypass
CKD: Chronic kidney disease
ICU: Intensive care unit
IRB: institutional review board
MACE: major cardiovascular and cerebral events
MFI: microcirculatory flow index
OPS: orthogonal polarized spectral
PPV: proportion of perfused vessels
PVD: perfused vessel density
RRT: Renal replacement therapy
SOFA: Sepsis-related Organ Failure Assessment
VS: Vasoplegic Syndrome

Declarations

Ethics approval and consent to participate

The institutional review board (IRB) of the University Hospital of Amiens (Comité de Protection des Personnes Nord-Ouest III, 80054 Amiens, France) approved the study (Registration number ID RDB: 2019-A02437-50 in February 2020). The Oxicard study was registered on the 17th of December 2019 on the ClinicalTrials.gov website with the trial identification number NCT04201119. We will obtain informed, written consent from all participants in the study.

Consent for publication

Not applicable.

Availability of data and materials

Data sharing is not applicable to this article because no datasets were generated or analyzed during the current study. However, data from the study will be made available at the end of the trial, on request.

Competing interests

The authors declare that they have no competing interests.

Funding

Oxicard trial was supported by Baxter Healthcare corporation (Baxter Acute Therapies grant program 2019 19CECACEU1002).

Author contributions

OAA and PH participated in the design of the study and helped to write the manuscript. GH, GT, CB, MD, YM and MG participated in the design of the study. MD will perform the statistical analysis. HD and YM helped to write the manuscript. All authors read and approved the final manuscript.

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Table 1. Endpoints and definitions

Endpoints	*Definitions*
Primary endpoint at day 1 after cardiac surgery	
Microcirculatory flow index	Microcirculatory flow index (MFI) measured by sublingual microcirculation device (Cytocam ®, Braedius Medical) at day 1 following cardiac surgery. A 20 s video sequence is recorded. A grid (4 quadrants) of the measurement area is made and the predominant type of flow is analyzed. The flux is noted from 0 (no flow) to 3 (continuous). The MFI is the average value of each the 4 quadrants, giving a total score from 0 to 3. The measurement is repeated for 4 sublingual areas.
Secondary endpoints	
Microcirculatory flow	Before CPB, at the end of CPB and at 6, 24 and 48 hours postoperatively with the following items: - MFI (microcirculatory flow index) - PPV (proportion of perfused vessels) - PVD (perfused vessel density) - HI (heterogeneity index)
Major Cardiovascular and Cerebral Event (MACE)	One of the following criterion (Definitions above): - Stroke - Myocardial infarction - Acute kidney injury - Mesenteric ischemia - Successful resuscitated cardiac arrest
Stroke	An embolic, thrombotic, or hemorrhagic cerebral event with persistent residual motor, sensory, or cognitive dysfunction (e.g., hemiplegia, hemiparesis, aphasia, sensory deficit, impaired memory) diagnosed on a cerebral scanner
Myocardial infarction	Myocardial infarction was diagnosed by the characteristics presentation, serial changes on 12-lead electrocardiographic suggesting infarction, and arise in

	cardiac markers, preferably cardiac troponins, with at least one value above the 99th percentile of the upper reference limit
Acute kidney injury	KDIGO guidelines Increase in serum creatinine of over 27 µmol/L within 48 hours or diuresis lower than 0.5 mL/kg/h
Mesenteric ischemia	Mesenteric ischemia confirmed by imaging or exploratory laparotomy and/or ischemic colitis confirmed by gastrointestinal endoscopy or exploratory laparotomy
Resuscitated cardiac arrest	Cessation of mechanical cardiac activity confirmed by the absence of clinical signs of blood flow.
In-hospital mortality	Mortality from surgery to hospital discharge
1-month hospital mortality	Mortality after surgery until 1-month follow-up
Cumulative catecholamine use	Cumulative dose of norepinephrine and dobutamine in resuscitation during stay in mg
SOFA score	Sepsis organ failure assessment
SAPS II	Simplified acute physiologic score assessment
Biomarkers of Glycocalyx degradation	Before CPB, at the end of CPB then at 6, 24 and 48 hours postoperatively. - Syndecan-1 - Heparan-sulphate - Hyaluronic acid Biomarkers will be assessed by ELISA test with dedicated kits.

Biomarkers of endothelial permeability	Before CPB, at the end of CPB then at 6, 24 and 48 hours postoperatively. - Angiopoietin 1 - Angiopoietin 2 - Soluble Tie2 receptor - VEGF Biomarkers will be assessed by ELISA test with dedicated kits.
Pro and anti-inflammatory cytokine	Before CPB, at the end of CPB then at 6, 24 and 48 hours postoperatively. - TNF alpha - IL1 beta - IL-10 - IL-6 - LPS - Endothelin Biomarkers will be assessed by ELISA test with dedicated kits.

CPB: Cardiopulmonary bypass**; MFI**: microcirculatory flow index; **PPV** (proportion of perfused vessels); **PVD** (perfused vessel density)

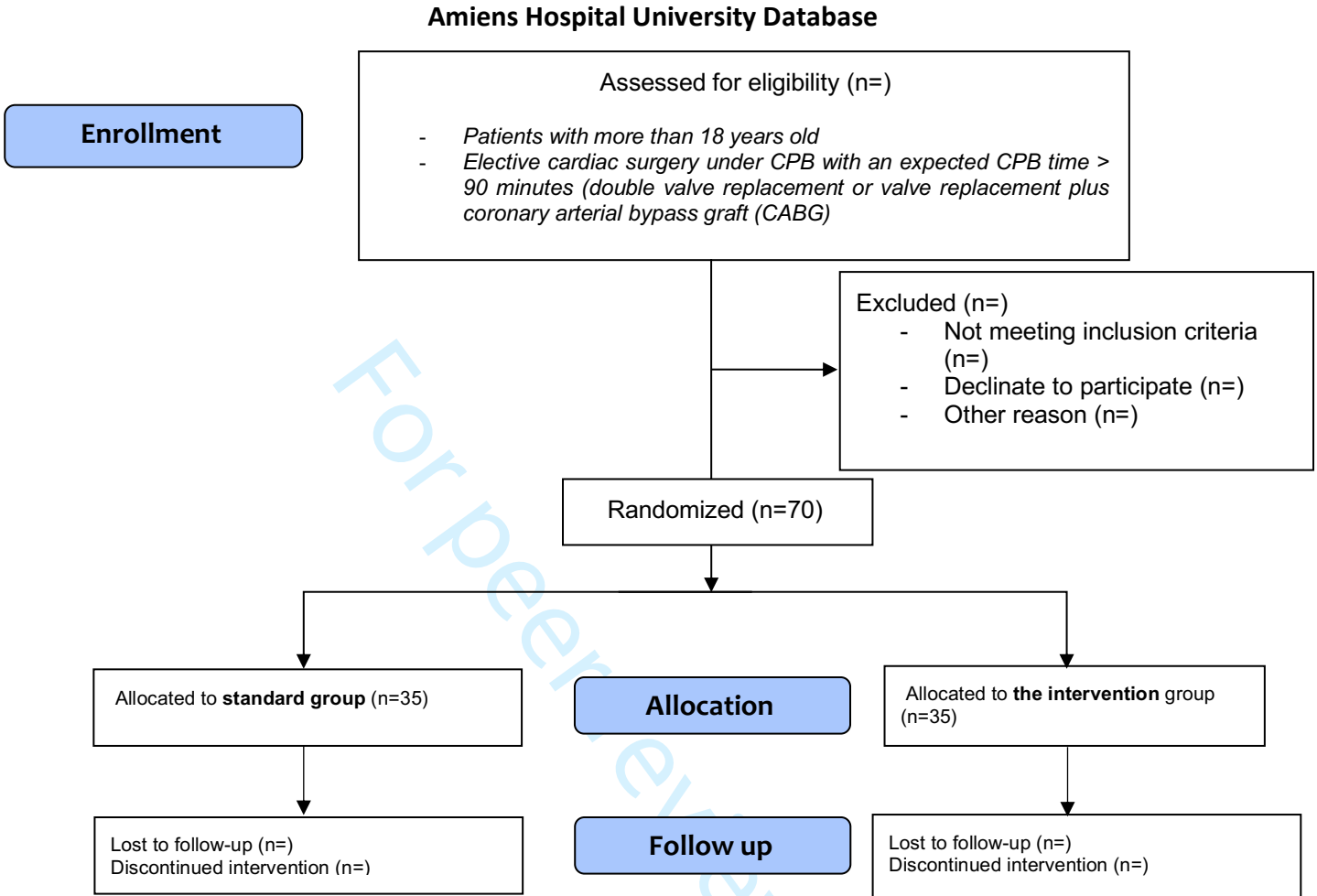
Figure legends

Figure 1. Consort diagram. **CPB:** Cardiopulmonary bypass,

Figure 2. Insertion of the Oxiris® membrane within the CPB. **CPB:** Cardiopulmonary bypass

Appendice : Informed consent given to participant and authorized surrogates

Figure 1. CONSORT diagram



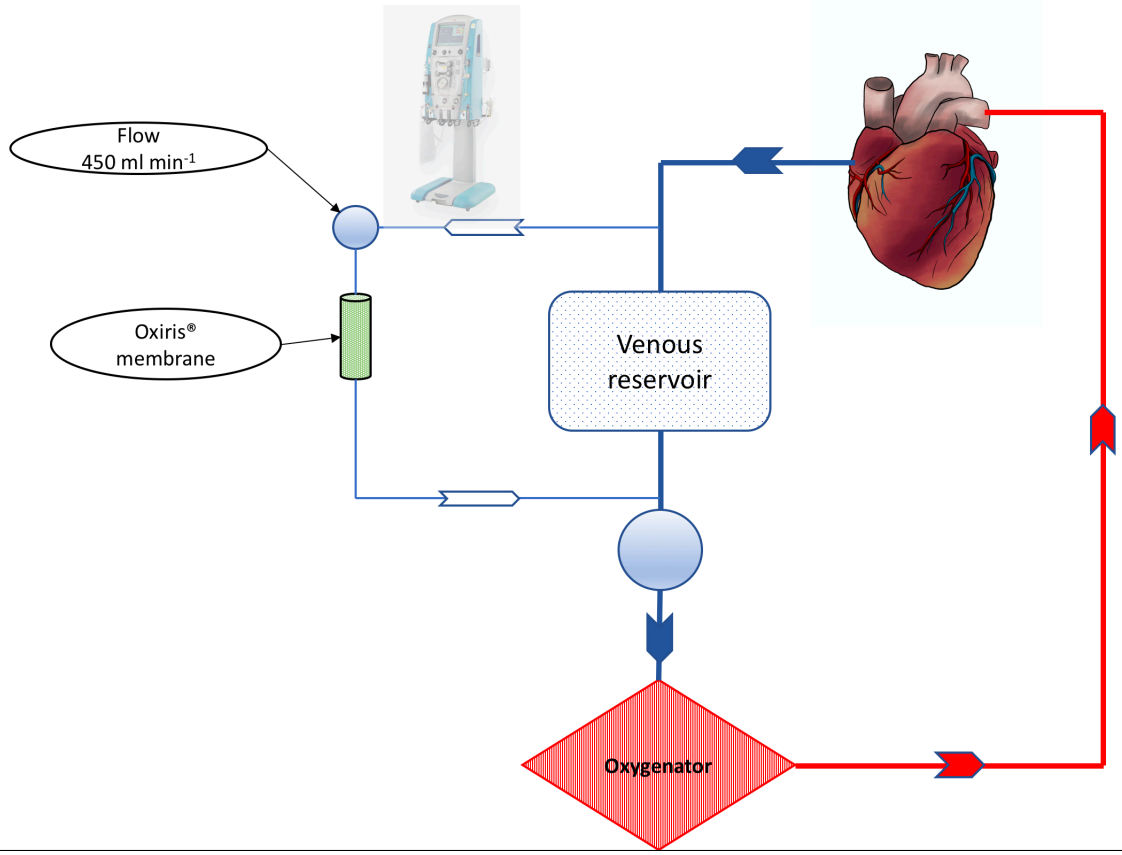
CPB: cardiopulmonary bypass;

Figure 2:

Insertion of the Oxiris® membrane within the cardiopulmonary bypass (CPB).

The Oxiris® membrane will be connected to the Prismaflex®.

The inflow of the Prismaflex® (and the Oxiris membrane) will be positioned between the venous line of CPB and the reservoir, and the outflow between the venous reservoir and the head of the pump.



Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

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

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Chan A-W, Tetzlaff JM, Altman DG, Laupacis A, Gøtzsche PC, Krleža-Jerić K, Hróbjartsson A, Mann H, Dickersin K, Berlin J, Doré C, Parulekar W, Summerskill W, Groves T, Schulz K, Sox H, Rockhold FW, Rennie D, Moher D. SPIRIT 2013 Statement: Defining standard protocol items for clinical trials. Ann Intern Med. 2013;158(3):200-207

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	2
Protocol version	#3	Date and version identifier	5
Funding	#4	Sources and types of financial, material, and other support	14
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	14

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	14
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	14
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	14
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	4
Objectives	#7	Specific objectives or hypotheses	5
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will	5

		perform the interventions (eg, surgeons, psychotherapists)	
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	6
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	6
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	7
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	8
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	8
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	10
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	10
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	11

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	6
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	6
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	6
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	6
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	8
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	8
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	8
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	9
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	10

Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	10
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and 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	7
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	10
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	6
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	6
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	5
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)	5
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	5
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	5
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	5

Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	14
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	11
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	6
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	11
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	11
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	11
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	18
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	9

BMJ Open

Effect of the oXiris® membrane on microcirculation after cardiac surgery under cardiopulmonary by-pass: study protocol for a randomized controlled trial (OXICARD Study).

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Effect of the oXiris® membrane on microcirculation after cardiac surgery under cardiopulmonary by-pass: study protocol for a randomized controlled trial (OXICARD Study).

Osama Abou-Arab[1], Pierre Huette[2], Guillaume Haye[3], Mathieu Guilbart[4], Gilles Touati[5], Momar Diouf [6], Christophe Beyls[7], Hervé Dupont[8], Yazine Mahjoub[9].

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Strengths and limitations of this study

- The trial will be a prospective, monocentric, randomized trial and will include 70 patients scheduled for cardiac surgery.
- We will include patients undergoing elective cardiac surgery under CPB with an expected CPB time > 90 minutes.
- The intervention that is being investigated is oXiris® membrane use during cardiac surgery under CPB.
- The primary endpoint will be the microcirculatory flow index (MFI) measured by sublingual microcirculation device at day 1 following cardiac surgery.
- The secondary endpoints will include major outcomes within the first 30 days, endothelium glycocalyx shedding variables, inflammatory cytokines and endothelial permeability biomarkers.

Abstract

Introduction

Cytokine storm and endotoxin release during cardiac surgery with cardiopulmonary bypass (CPB) have been related to vasoplegic shock and organ dysfunction. We hypothesized that early (during CPB) cytokines adsorption with oXiris® membrane for patients at high risk of inflammatory syndrome following cardiac surgery may improve microcirculation, endothelial function and outcomes.

Methods and analysis: The Oxicard trial is a prospective, monocentric trial, randomizing 70 patients scheduled for cardiac surgery. The inclusion criterion is patients with more than 18 years old undergoing elective cardiac surgery under CPB with an expected CPB time > 90 minutes (double valve replacement or valve replacement plus coronary arterial bypass graft (CABG)). Patients will be allocated to the intervention group (n=35) or the control group (n=35). In the intervention group, oXiris® membrane will be used on the Prismaflex device (Baxter) at blood pump flow of 450 ml min^{-1} during cardiac surgery under CPB. In the control group, cardiac surgery under CPB will be conducted as usual without oXiris® membrane. An intention-to-treat analysis will be performed. The primary endpoint will be the microcirculatory flow index (MFI) measured by sublingual microcirculation device at day 1 following cardiac surgery. The secondary endpoints will be other microcirculation variables at CPB end, 6 hours after CPB, at day 1 and at day 2. We also aim to evaluate the occurrence of major cardiovascular and cerebral events (MACE) (e.g., myocardial infarction, stroke, ischemic mesenteric, rescussited cardiac arrest, acute kidney injury) within the first 30 days. Cumulative catecholamine use, intensive care unit (ICU) length of stay, endothelium glycocalyx shedding parameters (syndecan-1, heparan-sulfate and hyaluronic acid), inflammatory cytokines (TNF alpha, IL1 beta, IL 10, IL 6, LPS, endothelin), and endothelial permeability biomarkers (angiopoietin 1, angiopoietin 2, Tie2 soluble receptor and VEGF) will also be evaluated.

Ethics and dissemination: Ethical approval has been obtained from the institutional review board (IRB) of the University Hospital of Amiens (Registration number ID RDB: 2019-A02437-50 in February 2020). Results of the study will be disseminated via peer-reviewed publications and presentations at national and international conferences.

Trial registration: NCT04201119. Registered on 17th of December 2019.

https://www.clinicaltrials.gov/ct2/show/NCT04201119?term=abou+arab&draw=2&rank=3

INTRODUCTION

A "sepsis-like syndrome" is often observed in postoperative cardiac surgery under cardiopulmonary-bypass (CPB), especially when CPB is over 90 minutes (double valve replacement or valve replacement plus coronary arterial bypass graft (CABG)). This condition is due to microcirculation disturbances induced by endothelial dysfunction. Indeed, extracorporeal circulation, associated with aortic clamping induces changes in the blood circulation with a so-called continuous circulation (unlike the usual pulsed circulation), leading to a heterogeneity of organ perfusion. The associated vasoplegic syndrome (SV) in post-operative cardiac surgery is due to acute circulatory failure, defined by persistent low blood pressure, requiring treatment with vasopressors [1] [2] [3]. From a pathophysiological point of view, SV is the result of a complex inflammatory cascade whose origin is multifactorial. This pro-inflammatory state activates complement, platelets and leukocytes which release vasoactive substances. Following CPB, impaired microcirculation is still common and can lead to true organ failure by decreasing oxygen delivery to tissues. Impaired microcirculation after bypass surgery has been well demonstrated with a decrease in microcirculatory flow to the lateral margin of the tongue. The use of sidestream or incident dark field/orthogonal polarized microscopy remains the reference method to clinically assess microcirculation [4][5]. The most easily accessible site at the patient's bedside is the microcirculation of the sublingual mucosa [6] [7] [8]. This pro-inflammatory state can be modulated with the removal of cytokines. The oXiris® (Baxter, IL, USA) membrane is an AN-69 membrane, surface-treated with polyethylenimine (PEI) and grafted with heparin. This property allows the adsorption of inflammatory cytokines and lipopolysaccharide (LPS) [9] [10]. The accepted indication is sepsis or septic shock requiring renal replacement therapy [11][12]. In cardiac surgery, there are no sufficient clinical data to recommend the use of the oXiris® membrane to reduce postoperative inflammatory syndrome. Some studies have demonstrated the feasibility of using the oXiris® membrane during cardiac surgery with CPB with effective removal of inflammatory cytokines [11][12]. However, to date, no studies have established the beneficial effect of cytokine removal on microcirculatory variables.

We hypothesized that the early use (during CPB) of cytokine adsorption with the oXiris® membrane in patients at high risk of inflammatory syndrome following cardiac surgery may improve microcirculation and hence, clinical outcomes.

METHODS AND ANALYSIS

Study design

This is a monocentric, prospective, randomized, study

Study population

The inclusion criteria are as follows:

- Patients with more than 18 years old
- Elective cardiac surgery under CPB with an expected CPB time > 90 minutes (double valve replacement or valve replacement plus coronary arterial bypass graft (CABG))
- Written informed consent from patient or next of kin.

The non-inclusion criteria are as follows:

- Missing informed consent.
- Patient under 18 years old.
- Planned hypothermia <32°C during CPB.
- Emergency surgery
- Acute infective endocarditis
- Immunosuppressive treatment or steroids (prednisone > 0.5mg/kg/day or equivalent)
- AIDS with a CD4 count of <200/mm^3
- Autoimmune disorder
- Transplant recipient
- Advances chronic Kidney Disease (CKD 4 or 5)
- Renal replacement therapy (RRT) in the last 90 days
- Known allergy to study device
- History of confirmed or suspected heparin-induced thrombocytopenia.
- Inclusion in other study within the last 30 days.
- Pregnancy

- Coexisting illness with a high probability of death (inferior to 6 months)

Study protocol

Randomization

Patients will be randomized into two parallel open-label group. Randomization will be carried out using Clinsight® software, implemented by a data manager. The result of randomization will be displayed as "standard group" or "intervention group». (Figure 1).

Intervention

In the intervention group, cardiac surgery will be conducted with the use of the oXiris® membrane connected to the CPB device.

The oXiris® membrane will be fitted to a dedicated Prismaflex® machine (Baxter,IL,USA) at the maximum flow rate recommended by the pharmaceutical laboratory at a blood flow rate of 450 ml.min^{-1}(Figure 2). The inflow of the Prismaflex® (and the Oxiris membrane) will be positioned between the venous line of CPB and the reservoir, and the outflow between the venous reservoir and the head of the CPB pump. In the standard group, cardiac surgery with CPB without the use of the oXiris® membrane. The conditions for performing CPB are those usually used for this type of surgery in the Anesthesia Department of the Amiens University Hospital, and do not differ from those that would be used outside the scope of this study.

For both arms, monitoring of sublingual microcirculation, primary and second endpoints will be assessed as defined in the data collection section.

There is no provision for ancillary and post-trial care.

Standard procedures

Patients will undergo cardiac surgery as usual and usual postoperative care will be provided. CPB circuit will be primed (1000 ml crystalloid and 500 ml colloid solution together with 5000 IE heparin) in accordance with institutional guidelines. CPB will be performed by using non-pulsatile flow at 2.5 l · min−1 · m−2, a non-heparin-coated circuit, and a membrane oxygenator (QuadroxTM, Maquet, Hirrlingen, Germany, or Capiox, Terumo, Eschborn, Germany). Blood transfusion will be performed in accordance with the Society of Thoracic Surgeons/ Society of Cardiovascular Anesthesiologists (STS-SCA) transfusion guidelines and administration of coagulation

factors will be based on blood samples[13]. Blood samples and all variables will be equally collected and registered in the control group with no modifications from CPB standard practice. The conduct of CPB will be standardized for the study with normothermia, hematocrit more than 20%, mean arterial pressure over 50 mmHg using vasopressor if required.

Standard of care will be given in the ICU after cardiac surgery to all study subjects. Especially for mechanical ventilation, nutrition, sedation, anticoagulation, and blood glucose control, therapy is based on local treatment protocols. In ICU, treatment with vasopressors, inotropes, and fluids is guided by hemodynamic monitoring using echocardiography to achieve a mean arterial pressure of 65 mmHg, a cardiac index over 2.5 ml min-1 m-2 and a diuresis> 0.5 ml kg-1 h-1. All patient will have a continuous cardiac output monitoring with a transpulmonary thermodilution device (Picco, Drager®). Hemodynamics time points assessment will be performed at ICU admission, 6 hours after, at day 1 and at day 2 after surgery.

Outcome measures

Primary endpoint

The endpoints and definitions are presented in Table 1.

The primary endpoint will be the microcirculatory flow using the MFI (microcirculatory flow index) measured by sublingual microcirculation device at day 1 following cardiac surgery.

Sublingual microcirculation will be visualized and measured using an incident dark field imaging device (Cytocam®, Breadius®, Amsterdam, The Netherlands) derived from the orthogonal polarized spectral (OPS) imaging. Five sequences of 20 seconds each from different adjacent sublingual areas will be recorded. Video sequences will be analyzed off-line by a single rater blinded to the time point of recording, to the study protocol, the surgical procedure, and the clinical status of the patient.

MFI will be assessed manually after dividing the screen in 4 quadrants. According to the classification of Boerma and colleagues [4], MFI values will be notified as follow: 0= no flow, 1= intermittent flow, 2 =sluggish flow, 3 =continuous flow. MFI value is the average value of the four quadrants.

Secondary endpoint

The secondary endpoints will be other microcirculatory flow variables: the perfused vessel density (PVD), the proportion of perfused vessels (PPV), the total vessel density and the De Backer score recorded by the sublingual microcirculation device (Cytocam®, Breadius®, Amsterdam, The Netherlands) and automatically calculated via a dedicated software (CytoCamtools® software (Braedius Medical, Huizen, the Netherlands) at different time points: prior to CPB, at the end of CPB, 6 hours after CPB, at day 1 and at day 2.

We also aim to evaluate the occurrence within the first 30 days of major cardiovascular and cerebral events (MACE) defined as the occurrence of at least one outcome among the following: successfully resuscitated cardiac arrest, stroke, acute kidney injury, myocardial infarction, mesenteric ischemia. Others outcomes will be in-hospital mortality and at 1-month, cumulative catecholamine use, intensive care unit stay (ICU) (days), hospital stay (days), SOFA score and SAPS II. Several biomarkers will be measured on blood sample. Endothelium glycocalyx shedding markers (syndecan-1, heparan-sulfate and hyaluronic acid), inflammatory cytokines (TNF alpha, IL1 beta, IL 10, IL 6, LPS), and endothelial permeability biomarkers (angiopoietin 1, angiopoietin 2, Tie2 soluble receptor, endothelin-1 and VEGF) will be also evaluated.

Data collection and outcome definitions

The following data will be collected: age (years), gender, body mass index (kg m^{-2}), medical history (coronary disease, peripheral vascular disease, stroke, smoking, diabetes, dyslipidemia, chronic obstructive pulmonary disease, logistic EuroSCORE[14], hypertension, chronic kidney disease, usual medication, surgery type (valve replacement, coronary bypass graft or combined surgery), preoperative left ventricular ejection fraction, baseline hemoglobin, duration of CPB and aortic clamp, intraoperative transfusion, and cumulative dose of vasoactive drugs (dobutamine, norepinephrine, ephedrine, phenylephrine).

Before and after CPB until day 2, the following data will be collected: systolic arterial pressure, diastolic arterial pressure, mean arterial pressure, heart rate, arterial blood gas variables (pH, PaO_2, $PaCO_2$, lactate, HCO_3), $ScVO_2$ (%), SOFA score, SAPS II score, vasoactive drug dose, and biological variables (creatinine, ASAT, ALAT, TP, TCA, platelet, Cardiac troponin, myoglobin). ICU and hospital stays (days) will be recorded. Endothelium glycocalyx shedding (syndecan-1, heparan-sulfate and

hyaluronic acid), inflammatory cytokine dosage (TNF alpha, IL1 beta, IL 10, IL 6, LPS, endothelin), and endothelial permeability biomarkers (angiopoietin 1, angiopoietin 2, Tie2 soluble receptor and VEGF) will be collected before CPB, at the end of CPB, 6 h after ending CPB, then at day 1 and day 2 after CPB.

Endpoints will be assessed after cardiac surgery. Adverse events will be declared and notified in the eCRFs.

Some secondary endpoints cannot be measured blindly and may be influenced by the treatment group. An independent observer blind from treatment group will be in charge of collecting data. The primary outcome and biological data will be collected blind to treatment group.

Standard definitions of postoperative outcomes established by the European Society of Anesthesia were used [15]. Cardiac arrest is defined as the cessation of cardiac mechanical activity, as confirmed by the absence of circulation signs. Stroke is defined as an embolic, thrombotic, or hemorrhagic cerebral event with persistent residual motor, sensory, or cognitive dysfunction (e.g. hemiplegia, hemiparesis, aphasia, sensory deficit, impaired memory) diagnosed on a cerebral scanner. Acute kidney injury is defined according to Kidney Disease Improving Global Outcomes (KDIGO) criteria as an increase in serum creatinine of over 27 µmol/L within 48 h or diuresis lower than 0.5 mL/kg/h [16]. Myocardial injury is diagnosed by the characteristics presentation, serial changes on 12-lead electrocardiographic suggesting infarction, and arise in cardiac troponin, with at least one value above the 99th percentile of the upper reference limit [17]. Mesenteric ischemia will be confirmed by imaging or exploratory laparotomy and ischemic colitis will be confirmed by gastrointestinal endoscopy or exploratory laparotomy.

Intention-to-treat analysis

Patients with serious adverse events will be analyzed according to their assigned group following the intention-to-treat principle.

Statistical method and Sample size calculation

According to a recent study we predict a baseline mean MFI of 2.8 with a standard deviation (SD) of 0.5 [5].

Admitting that the standard deviation of MFI at D1 is 0.5 in both groups, we calculated a sample size of 70 patients to show a difference in MFI of 0.4 at D1 after surgery in a 2-sided test with a type 1 error of 0.05 and a power of 90%. Approximately 450 cardiac surgery procedures with bypass surgery are performed per year at Amiens University Hospital. If we consider that 30% of patients will be eligible, this represents a potential inclusion of 130 patients per year. Taking into account possible non-inclusions due to vacations and operating room closures, a total inclusion time of 7 months is expected for a total of 70 patients. 35 patients in each group (standard and interventional groups).

Primary end point will be compared by a Student test or Wilcoxon-Mann-Whitney test as appropriate. Secondary end points will be assessed using an ANOVA test for repeated measures. MACE and mortality rate will be compared with a chi-2 square test. ICU and hospital stay will be compared using a Student test. Cumulative event curves will be estimated with the Kaplan-Meier procedure (censored at 30 days). Variables or parameters between usual and intervention groups will be compared with a Student test, a Wilcoxon-Mann-Whitney test, a chi-2 square or a Fischer exact test as appropriate. A p value of 0.05 will be considered as significant. No intermediate analysis is planned in the trial.

Data management and monitoring

Registration

Data will be collected and registered using electronic case report forms (eCRFs) by a dedicated local research technician. A research coordinator will centralize and verify the data.

Record keeping

Consent forms and eCRFs will be retained for 15 years at the University Hospital of Amiens in accordance with French law.

Study organization

The study promotion is performed by the University Hospital of Amiens, France.

Duration and timeline

Patients from Amiens university hospital can be included during a 2-year period beginning from July 2020.

The processes of developing the protocol, obtaining approval from the ethical committee, obtaining financial support, and developing the eCRFs occurred in 2019. The database should be closed after all participants have been included, followed by data analysis, manuscript writing and submission for publication.

ETHICS AND DISSEMINATION

The institutional review board (IRB) of the University Hospital of Amiens (Comité de Protection des Personnes Nord-Ouest III, 80054 Amiens, France) approved the study (Registration number ID RDB: 2019-A02437-50 in February 2020). The Oxicard study will be conducted in accordance with the Declaration of Helsinki and French law on clinical research [18] and was registered on the 17th of December 2019 on the ClinicalTrials.gov website with the trial identification number NCT04201119. Oxicard trial follows CONSORT Statement - CONSORT diagram is given in Figure 1 [19]. Written informed consent will be obtained from all participants or next of kin.

Authors will be involved in disseminating research findings (through attending conferences and co-authoring results 'papers).

Patients or public involvement

Patients or the public will not be involved in the design, or conduct, or reporting, or dissemination plans of our research. Written informed consent will be obtained from all participants or next of kin.

Discussion

The hypothesis is that early use (during CPB) of cytokine adsorption with the oXiris® membrane in patients at high risk of VS following cardiac surgery may improve microcirculation and endothelial function.

Postoperative period following cardiac surgery is associated with an inflammatory state leading to side effects such as vasoplegia and major adverse events[20]. Clinical and experimental data support a link between inflammation with cytokine production and endothelial cell injury [21]. Microcirculatory impairment have also been observed after cardiac surgery [8][22] [23].

Despite normal macrocirculatory variables, microcirculatory dysfunction resulting in functional oxygen shunting can lead to impairment in oxygen delivery and thus, to organ dysfunction and mortality [24].

Several studies have investigated cytokine adsorption in vitro. Moreover, it has been shown that oXiris® membrane reduces the concentration of cytokines after CPB and during sepsis [9][10]. However, to date, no studies have been performed on the effect of cytokine adsorption on endothelial function and microcirculation.

We hypothesize that systemic microcirculation, assessed by sublingual microcirculation, may be improved when an adsorbent membrane is used to reduce the amount of circulating cytokines.

A pilot study to evaluate the cytokine adsorption effect is an important step before carrying out a larger prospective randomized study with clinical endpoints.

Nevertheless, we will also assess major cardiovascular and cerebral events (stroke, acute kidney injury and myocardial infarction …) in order to evaluate the impact of improved microcirculation on organ dysfunction. In the event of a positive effect, a multicenter randomized trial will be conducted using morbidity and mortality as primary endpoints.

We have privileged sublingual microcirculatory measurement at day 1as a primary endpoint. Measurement at H6 may be difficult when the patient is awakening. 6 hours after the end of cardiopulmonary by-pass, some patients may still be under sedation and some may not. We will adjust the results on the patient's state of awakening in order to avoid measurement bias

Trial status

The trial is not yet recruiting.

List of abbreviations

AIDS: Acquired-immune-deficiency-syndrome
CABG: coronary arterial bypass graft
CPB: Cardiopulmonary bypass
CKD: Chronic kidney disease
ICU: Intensive care unit
IRB: institutional review board
MACE: major cardiovascular and cerebral events
MFI: microcirculatory flow index
OPS: orthogonal polarized spectral
PPV: proportion of perfused vessels
PVD: perfused vessel density
RRT: Renal replacement therapy
SOFA: Sepsis-related Organ Failure Assessment
VS: Vasoplegic Syndrome

Declarations

Ethics approval and consent to participate

The institutional review board (IRB) of the University Hospital of Amiens (Comité de Protection des Personnes Nord-Ouest III, 80054 Amiens, France) approved the study (Registration number ID RDB: 2019-A02437-50 in February 2020). The Oxicard study was registered on the 17th of December 2019 on the ClinicalTrials.gov website with the trial identification number NCT04201119. We will obtain informed, written consent from all participants in the study.

Consent for publication

Not applicable.

Availability of data and materials

Data sharing is not applicable to this article because no datasets were generated or analyzed during the current study. However, data from the study will be made available at the end of the trial, on request.

Competing interests

The authors declare that they have no competing interests.

Funding

Oxicard trial was supported by Baxter Healthcare corporation (Baxter Acute Therapies grant program 2019 19CECACEU1002).

Author contributions

OAA and PH participated in the design of the study and helped to write the manuscript. GH, GT, CB, MD, YM and MG participated in the design of the study. MD will perform the statistical analysis. HD and YM helped to write the manuscript. All authors read and approved the final manuscript.

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Table 1. Endpoints and definitions

Endpoints	*Definitions*
Primary endpoint at day 1 after cardiac surgery	
Microcirculatory flow index	Microcirculatory flow index (MFI) measured by sublingual microcirculation device (Cytocam ®, Braedius Medical) at day 1 following cardiac surgery. A 20 s video sequence is recorded. A grid (4 quadrants) of the measurement area is made and the predominant type of flow is analyzed. The flux is noted from 0 (no flow) to 3 (continuous). The MFI is the average value of each the 4 quadrants, giving a total score from 0 to 3. The measurement is repeated for 4 sublingual areas.
Secondary endpoints	
Microcirculatory flow	Before CPB, at the end of CPB and at 6, 24 and 48 hours postoperatively with the following items: - MFI (microcirculatory flow index) - PPV (proportion of perfused vessels) - PVD (perfused vessel density) - HI (heterogeneity index)
Major Cardiovascular and Cerebral Event (MACE)	One of the following criterion (Definitions above): - Stroke - Myocardial infarction - Acute kidney injury - Mesenteric ischemia - Successful resuscitated cardiac arrest
Stroke	An embolic, thrombotic, or hemorrhagic cerebral event with persistent residual motor, sensory, or cognitive dysfunction (e.g., hemiplegia, hemiparesis, aphasia, sensory deficit, impaired memory) diagnosed on a cerebral scanner
Myocardial infarction	Myocardial infarction was diagnosed by the characteristics presentation, serial changes on 12-lead electrocardiographic suggesting infarction, and arise in

	cardiac markers, preferably cardiac troponins, with at least one value above the 99th percentile of the upper reference limit
Acute kidney injury	KDIGO guidelines Increase in serum creatinine of over 27 µmol/L within 48 hours or diuresis lower than 0.5 mL/kg/h
Mesenteric ischemia	Mesenteric ischemia confirmed by imaging or exploratory laparotomy and/or ischemic colitis confirmed by gastrointestinal endoscopy or exploratory laparotomy
Resuscitated cardiac arrest	Cessation of mechanical cardiac activity confirmed by the absence of clinical signs of blood flow.
In-hospital mortality	Mortality from surgery to hospital discharge
1-month hospital mortality	Mortality after surgery until 1-month follow-up
Cumulative catecholamine use	Cumulative dose of norepinephrine and dobutamine in resuscitation during stay in mg
SOFA score	Sepsis organ failure assessment
SAPS II	Simplified acute physiologic score assessment
Biomarkers of Glycocalyx degradation	Before CPB, at the end of CPB then at 6, 24 and 48 hours postoperatively. - Syndecan-1 - Heparan-sulphate - Hyaluronic acid Biomarkers will be assessed by ELISA test with dedicated kits.

Biomarkers of endothelial permeability	Before CPB, at the end of CPB then at 6, 24 and 48 hours postoperatively. - Angiopoietin 1 - Angiopoietin 2 - Soluble Tie2 receptor - VEGF Biomarkers will be assessed by ELISA test with dedicated kits.
Pro and anti-inflammatory cytokine	Before CPB, at the end of CPB then at 6, 24 and 48 hours postoperatively. - TNF alpha - IL1 beta - IL-10 - IL-6 - LPS - Endothelin Biomarkers will be assessed by ELISA test with dedicated kits.

CPB: Cardiopulmonary bypass**; MFI**: microcirculatory flow index; **PPV** (proportion of perfused vessels); **PVD** (perfused vessel density)

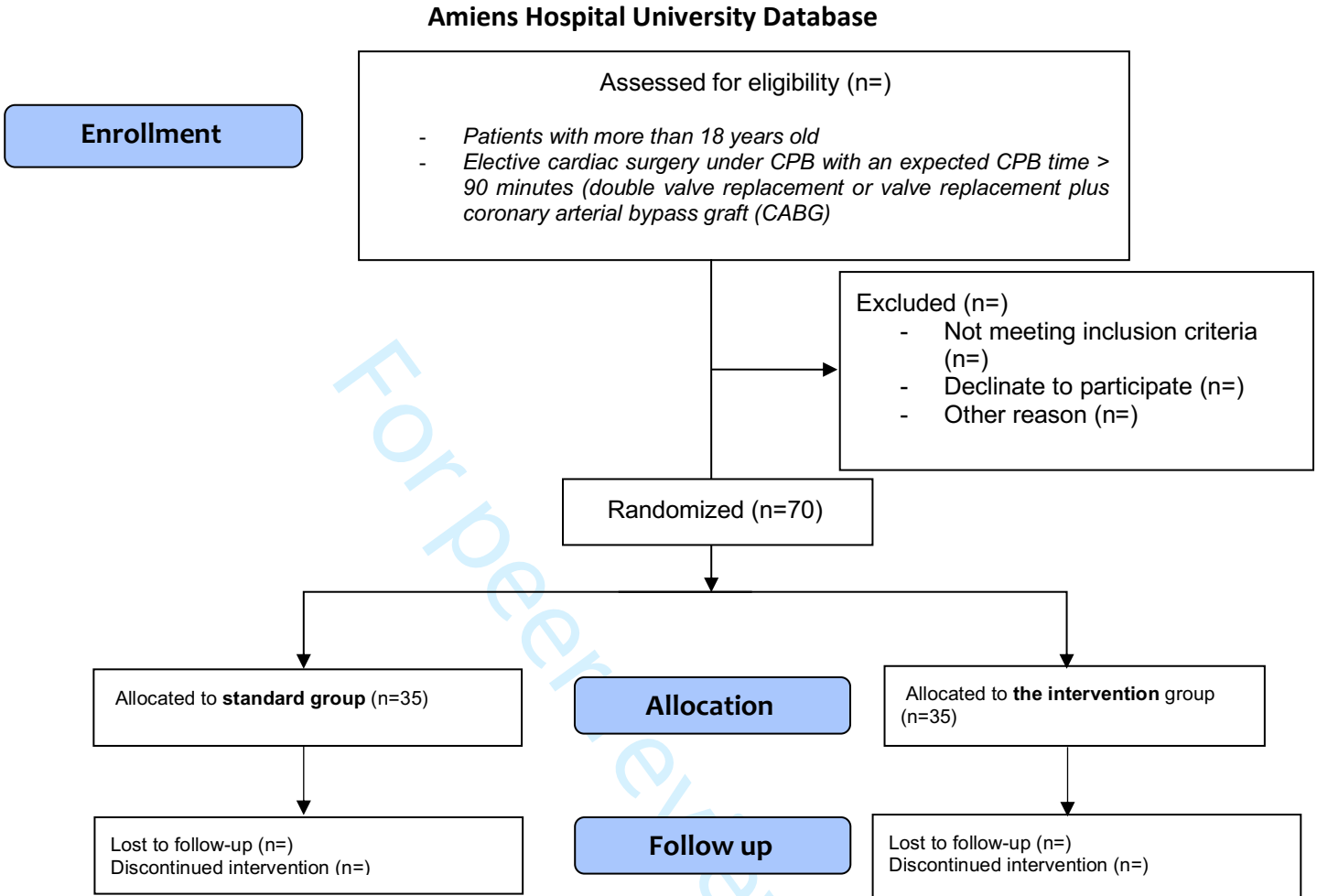
Figure legends

Figure 1. Consort diagram. **CPB:** Cardiopulmonary bypass,

Figure 2. Insertion of the Oxiris® membrane within the CPB. **CPB:** Cardiopulmonary bypass

Appendice: Informed consent given to participant and authorized surrogates

Figure 1. CONSORT diagram



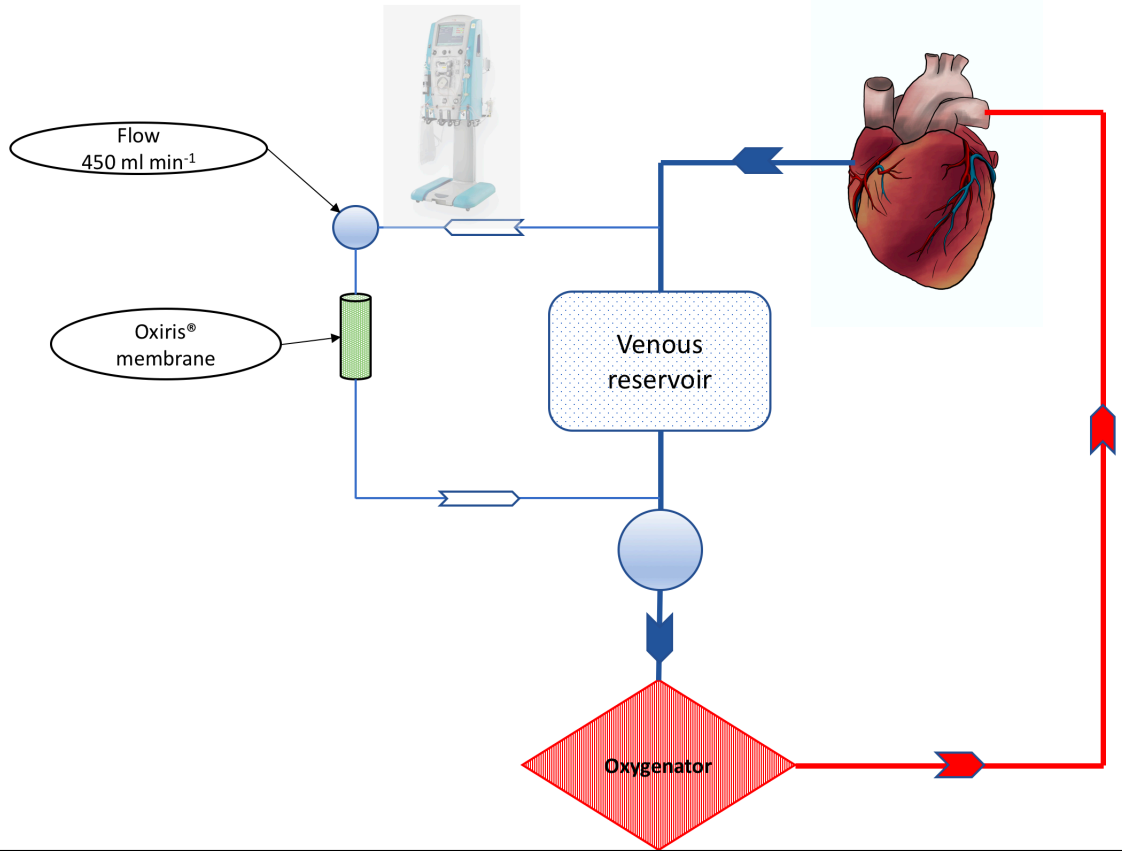
CPB: cardiopulmonary bypass;

Figure 2:

Insertion of the Oxiris® membrane within the cardiopulmonary bypass (CPB).

The Oxiris® membrane will be connected to the Prismaflex®.

The inflow of the Prismaflex® (and the Oxiris membrane) will be positioned between the venous line of CPB and the reservoir, and the outflow between the venous reservoir and the head of the pump.



Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

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

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

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

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

Chan A-W, Tetzlaff JM, Altman DG, Laupacis A, Gøtzsche PC, Krleža-Jerić K, Hróbjartsson A, Mann H, Dickersin K, Berlin J, Doré C, Parulekar W, Summerskill W, Groves T, Schulz K, Sox H, Rockhold FW, Rennie D, Moher D. SPIRIT 2013 Statement: Defining standard protocol items for clinical trials. Ann Intern Med. 2013;158(3):200-207

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	2
Protocol version	#3	Date and version identifier	5
Funding	#4	Sources and types of financial, material, and other support	14
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	14

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	14
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	14
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	14
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	4
Objectives	#7	Specific objectives or hypotheses	5
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will	5

		perform the interventions (eg, surgeons, psychotherapists)	
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	6
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	6
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	7
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	8
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	8
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	10
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	10
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	11

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	6
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	6
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	6
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	6
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	8
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	8
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	8
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	9
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	10

Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	10
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and 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	7
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	10
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	6
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	6
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	5
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)	5
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	5
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	5
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	5

Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	14
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	11
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	6
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	11
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	11
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	11
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	18
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	9