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Keywords:	Nitric Oxide, Hemolysis, Acute Kidney Injury, Cardiopulmonary Bypass, Endothelial Dysfunction

TITLE PAGE

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Author Contributions: Authorship for this trial will be given to key personnel involved in trial design, recruitment, data collection, and data analysis. There are no publication restrictions. LB, WZ, JB, TBT, NR, EB, TS, DF, ER, FM, and FI were responsible for conceptualizing trial design. LB managed patient safety protocol. FM, SS, FZ, CX, GL, and TSL are responsible for recruitment, enrollment, and data collection. All authors have critically revised the study protocol and approved the final version. All authors agree to be accountable for the accuracy and integrity of all aspects of this trial.

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Article Summary:

Strengths and limitations of this study:

- Circulating plasma hemoglobin during and following hemolysis causes depletion of vascular nitric oxide, vasoconstriction, and acute kidney injury (AKI). Supplementation with nitric oxide gas during and after surgery requiring prolonged cardiopulmonary bypass might be most beneficial to those patients with endothelial dysfunction unable to produce NO due to impaired endothelial nitric oxide synthase (eNOS) activity.

- At our institution, the “Kidney Disease: Improving Global Outcomes” (KDIGO) guidelines for renal protection[1] are a standard of care. Thus, the number of confounding variables resulting from the liberal use of nephrotoxic agents, the preoperative variability in serum creatinine level and the perioperative optimization of hemodynamics and volume status is minimized.
- Results from this study could possibly be generalized to other clinical scenarios of intravascular hemolysis and vascular depletion of nitric oxide.
- This trial is designed to recruit patients from a single academic institution (Massachusetts General Hospital).

ABSTRACT

Introduction: Postoperative acute kidney injury (AKI) is a common complication in cardiac surgery. Levels of intravascular hemolysis are strongly associated with postoperative AKI and with prolonged (>90 minutes) use of cardiopulmonary bypass (CPB). Ferrous plasma hemoglobin released into the circulation acts as a scavenger of nitric oxide (NO) produced by endothelial cells. Consequently, the vascular bioavailability of NO is reduced, leading to vasoconstriction and impaired renal function. In patients with cardiovascular risk factors the endothelium is dysfunctional and cannot replenish the NO deficit. A previous clinical study in young cardiac surgical patients with rheumatic fever, without evidence of endothelial dysfunction, showed that supplementation of NO gas decreases AKI by converting ferrous plasma hemoglobin to ferric methemoglobin, thus preserving vascular NO. In this current trial we hypothesized that 24 hours administration of NO gas will reduce AKI following CPB in patients with endothelial dysfunction.

Methods and analysis: This is a double-blind, single-center, randomized (1:1) controlled, parallel-arm superiority trial that includes patients with endothelial dysfunction, stable kidney function, and who are undergoing cardiac surgery procedures with an expected CPB duration >90 minutes. Patients are randomized to receive either nitrogen (N_2, control group) or 80 parts-per-million NO (intervention group). Test gases (N_2 or NO) are delivered during CPB and for 24 hours after surgery. The primary study outcome is the occurrence of AKI among study groups. Key secondary outcomes include AKI severity, occurrence of renal replacement therapy, major adverse kidney events at 6 weeks after surgery, and mortality. We are recruiting 250 patients, allowing detection of a 35% AKI relative risk reduction, assuming a two-sided error of 0.05.

Ethics and dissemination: The Partners Human Research Committee approved this trial. Recruitment began in February 2017. Dissemination plans include presentations at scientific

conferences, scientific publications, and advertising flyers and posters at Massachusetts General Hospital.

TEXT

Introduction

Acute kidney injury (AKI) is a common and serious complication after cardiac surgery procedures requiring prolonged (>90 minutes) cardiopulmonary bypass (CPB)[2–4]. Because post-cardiac surgical AKI is associated with increased risk of chronic kidney disease (CKD) and mortality[5–7], multiple interventions have been tested in large multicenter trials, but none have been successful at reducing AKI[8–11].

There are two major categories of risk factors for post-surgical AKI. The first is the patient's cardiovascular comorbidities that are associated with impaired endothelial function. These include obesity, diabetes, atherosclerosis, peripheral vascular disease, hyperlipidemia, smoking, and hypertension[3,12]. The second is the extent of CPB-associated hemolysis[13–16]. During hemolysis, plasma hemoglobin (Hb) is released into the circulation, and depletes plasma nitric oxide (NO)[17,18]. NO is a potent mediator of vasodilation and regulates blood flow to tissues[19]. In patients with endothelial dysfunction, the activity of endothelial NO synthase (eNOS), the enzyme responsible for NO production, is impaired and is unable to replenish the NO consumed by plasma Hb[20–22].

In an animal model of free water-induced intravascular hemolysis, Minneci et al. showed that hemolysis resulted in a reduction of creatinine clearance. The negative effects of hemolysis on renal function were attenuated by the inhalation of 80 parts-per-million (ppm) NO gas. NO oxidized plasma Oxy-Hb (Fe^{2+}) to ferric methemoglobin (Met-Hb, Fe^{3+}), thereby decreasing plasma NO consumption[17]. In a recent randomized controlled trial, administration of 80ppm NO gas for 24 hours, starting at the onset of CPB, was found to decrease postoperative AKI in a population of young Chinese cardiac surgical patients (average age 48), with no risk factors for endothelial dysfunction, who were undergoing cardiac valve replacement due to rheumatic heart disease[18]. NO administration also improved long-term outcomes such as Major Adverse Kidney

Events (MAKE) and reduced stage 3 CKD for up to 1-year after surgery. However, it is still undetermined whether NO gas exerts similar, or even additional, renal protective effects in patients with endothelial dysfunction commonly treated in the United States.

Methods and analysis

Trial design

In this double-blind, single-center, randomized (1:1) controlled, parallel-arm superiority trial (RCT), we hypothesized that 24-hour administration of NO gas starting from the initiation of CPB will decrease AKI by converting Oxy-Hb to Met-Hb in patients with signs and symptoms of endothelial dysfunction suggestive of impaired activity of eNOS (primary outcome).

After screening, consented patients who meet the enrollment criteria are randomized to receive either 80ppm NO (intervention group, n=125) or Nitrogen (N_2, control group, n=125) for 24 hours. The test gas is administered via the oxygenator during CPB, then by inhalation once mechanical ventilation is resumed. When the patient is extubated, the test gas is delivered by high flow nasal cannula. On the day of surgery, after the insertion of a pulmonary artery catheter (PAC), patients are stratified by preoperative mean pulmonary artery pressure (i.e. mPAP <30 mmHg or mPAP ≥30 mmHg) then allocated into the two study groups. Patients with mPAP ≥40 mmHg are not included in this study because NO or other pulmonary vasodilators might be indicated as part of the medical treatment during the perioperative period. Study outcomes are assessed during the hospital stay, at a 6-week follow-up visit, and by phone at 90 days and 1 year after the surgical procedure.

In order to avoid postoperative AKI, at our institution the "Kidney Disease: Improving Global Outcomes (KDIGO) guidelines" are the standard of care in all cardiac surgical patients[1]. The implementation of these guidelines has been shown to limit the incidence and severity of CPB-associated AKI[23]. Per these guidelines, patients do not receive any nephrotoxic agents such as intravenous contrast agents or antibiotics such as vancomycin[24] during the first 48 hours after surgery. Patients receive close monitoring of serum creatinine and hourly urine output, and avoid intraoperative and postoperative hyperglycemia for at least 72 hours after surgery. Hemodynamics are closely monitored to maintain adequate cardiac output (CO) and blood pressure by using intraoperative transesophageal echocardiography (TEE) in combination with the PAC and a radial artery catheter[1,23]. The pulmonary and radial artery catheters are removed only after the patient is discharged from the intensive care unit (ICU) and after completion of test gas treatment.

The study enrollment opened in February 2017 and the first patient entered the trial in June 2017. The study team plans to enroll 250 patients over a period of 3 years. The study design is depicted in Figure 1.

Eligibility criteria

The study population includes men >40 years old and women >50 years old who have signs and symptoms of endothelial dysfunction, have stable kidney function in the 3 months prior to surgery, are undergoing cardiac surgery procedures with expected prolonged CPB (>90 minutes), and are not currently enrolled in other research studies. The presence of endothelial dysfunction is evaluated by using a brief questionnaire that integrates medical history with clinical and laboratory data that has previously been associated with endothelial dysfunction (Figure 2: Screening questionnaire to detect endothelial dysfunction)[25–34]. Eligible patients are those undergoing a scheduled procedure or patients who are already admitted to the hospital.

Key exclusion criteria include estimated glomerular filtration rate (eGFR) <30 ml/min/1.73m^2, mPAP ≥40 mmHg and intravenous (I.V.) contrast infusion within 48 hours before surgery. A complete list of enrollment criteria is provided in Table 1.

Intervention

Perioperative management

Perioperative management including the implementation of the KDIGO guidelines has been shown to decrease the incidence of post-cardiac surgical AKI[23]. These guidelines recommend the avoidance of nephrotoxic agents, the close monitoring of serum creatinine and urine output, the avoidance of hyperglycemia, the consideration of alternatives to radiocontrast agents, and a close hemodynamic monitoring to maintain adequate cardiac output, blood pressure and peripheral oxygen delivery. To reduce AKI incidence related to associated factors during prolonged CPB, the KDIGO guidelines have been implemented at our institution as a standard of care in all patients undergoing cardiac surgery.

Anesthesia management of patients enrolled in the study is performed according to the hospital standard of care and using standardized protocols[35]. Before entering the operating room (OR), patients are pre-medicated with 2-5 mg of midazolam. Prophylactic antibiotics (cefazolin 2-3 g or vancomycin 1-2 g) are administered through a peripheral venous catheter (Introcan Safety® IV Catheter, B Braun). These are given during the hour before the surgical incision. In the OR, electrocardiography (EKG) and peripheral oxygen saturation (SpO_2) are continuously recorded. A radial arterial catheter (Arrow® Seldinger Arterial Catheter) is placed for continuous blood pressure monitoring and blood sampling. Patients are then pre-oxygenated at an inspired oxygen concentration of 100% for 3 minutes. Anesthesia is then induced with an I.V. bolus injection of 4-6 µg/kg of fentanyl and either propofol 2 mg/kg or etomidate 0.1 mg/kg. Rocuronium 1.2 mg/kg or cisatracurium 0.2 mg/kg are used for paralysis prior to oro-tracheal

intubation. Medication doses are adjusted in elderly patients. After intubation, patients are connected to the anesthesia machine, and mechanical ventilation is delivered in volume-controlled mode with a tidal volume of 6 ml/kg. Respiratory rate is titrated to maintain a partial pressure of carbon dioxide ($PaCO_2$) between 35-45 mmHg. The inspired fraction of oxygen (FiO_2) is titrated according to the partial pressure of oxygen (PaO_2), which is measured with arterial blood gas (ABG) analysis. The TEE probe (X7-2t 3D ultrasound probe, Philips) is inserted by the cardiac-anesthesia team for real-time intraoperative feedback to cardiac surgeons on (I) right and left ventricular function, (II) the mechanical stability and the function of repaired/replaced heart valves, and (III) the detection of air emboli/clots. The neck, chest, and abdomen are prepped and draped in a sterile fashion. A urinary catheter is positioned for hourly urinary output monitoring. To determine intraoperative and postoperative effects of NO on hemodynamics, a PAC (Edwards Lifescience, Irvine, CA) is placed in all patients for continuous hemodynamic monitoring of central venous pressure (CVP), pulmonary artery pressure (PAP), and cardiac index (CI). Electrodes for SEDLine brain function monitoring (Masimo®, Irvine, CA) are positioned on the forehead of each patient to assess the depth of anesthesia by displaying frontal cortex electroencephalogram waveforms and measuring the patient state index (PSI)[36]. The level of anesthesia is maintained by isoflurane administration (0.8-1.2 minimum alveolar concentration) and adjusted based on hemodynamics, while sustaining a PSI score between 30 and 50. Paralysis is maintained throughout the entire procedure by continuous infusion of rocuronium 4-10 µg/kg/min or cisatracurium 1-3 µg/kg/min. When clinically indicated, inotropes or vasopressors are administrated to maintain a mean arterial pressure (MAP) >65 mmHg and a CI >2.2 l/min/m^2.

The perfusion equipment utilized at Massachusetts General Hospital consists of Stockert S5 (LivaNova, Mirandola, Italy) heart-lung machines, CardioQuip (CardioQuip, Bryan, TX, USA)

cooler-heaters, the LivaNova Xtra autologous cell salvage system and Medtronic HMS Plus Hepcon system (Medtronic, Minneapolis, MN, USA).

The CPB circuit is primed with 1600 ml Ringer's Lactate and recirculated for priming and air removal. Prior to cannulation, the patient is fully heparinized to a target ACT of no less than 400 seconds and a target heparin concentration of no less than 2.0 I.U./ml. The loading dose of heparin is calculated by the Hepcon device and subsequent ACT and heparin concentration assays are performed on the same system. The cannulation strategy may be central, peripheral or a combination of both depending on the type of surgery (coronary/valve vs. aortic), level of urgency, surgical approach (median sternotomy vs. thoracotomy), patient specific variables such as body habitus, previous cardiac surgical history, vascular disease and vascular anatomic anomalies. Most commonly, an arterial outflow cannula is placed in the distal ascending aorta and venous drainage is achieved by placing a multi-stage venous cannula in the right atrium via the inferior vena cava (IVC). The aortic root is typically cannulated with a catheter to deliver antegrade cardioplegia as well as to decompress the left ventricle. Retrograde catheters are commonly placed in the coronary sinus to maximize myocardial protection during the aortic cross clamp.

Once fully cannulated and prior to CPB, 500ml to 1000ml of prime volume may be removed from the CPB circuit via retrograde autologous priming (RAP) and/or venous antegrade priming (VAP) to reduce the crystalloid burden on the patient upon initiation of CPB. Once the patient is placed on CPB hypothermia is initiated to an extent dictated by type of surgery and surgeon's preference. Mild hypothermia (temperature maintained between 32-35°C) is most common for coronary and valve operations whereas circulatory arrest cases call for deeper hypothermia (temperature maintained between 18-24°C).

Aortic occlusion is achieved by application of an aortic cross clamp and myocardial protection is achieved by administration of either Del Nido cardioplegia[37] or a traditional 4:1 (blood:crystalloid) mixture at hypothermia into the aortic root (antegrade) and the coronary sinus

(retrograde). Diastolic arrest is maintained by intermittent doses of cardioplegia every 20-30 minutes.

Blood flow rates while on CPB are maintained at a cardiac index (CI) of 2.4 l/min/m^2 or greater, a MAP of 65-75 mmHg and a urinary output of greater than 0.5 ml/kg/hr. To achieve these MAP goals phenylephrine may be titrated to effect and diuretics such as furosemide (10 mg bolus) may be given. Sweep gas flow and FiO_2 are regulated to maintain a PaO_2 of 150-250 mmHg, $PaCO_2$ of 35-45 mmHg and a mixed venous oxygen saturation (SvO_2) greater than 65%. Arterial and venous blood gases are drawn every 30 minutes and analyzed by the hospital laboratory. Ultrafiltration with a LivaNova hemoconcentrator (LivaNova, Mirandola, Italy) may be performed with or without maintaining zero balance fluid administration for the purposes of normalizing potassium levels (target K^+ 3.5-5.5 mmol/l) and to increase hematocrit (target hematocrit (HCT) greater than 21%), respectively. In the event ultrafiltration is inadequate to increase HCT to greater than 21% packed red blood cells or red blood cells processed by intraoperative autotransfusion may be administered via the LivaNova Xtra autologous cell salvage system. Target blood glucose levels of 100-180 mg/dL are maintained by administration of IV insulin (bolus 10 I.U. and continuous infusion of 2 I.U./hour) when the glucose level exceeds 180 mg/dL.

Once the procedure is complete, the heart and aorta are de-aired and the aortic cross clamp is removed. Temporary epicardial pacing leads are placed, the surgical site is policed for bleeding and the patient is weaned from CPB. Upon satisfactory termination from CPB, the cannulas are removed and the patient is administered protamine sulfate to reverse heparin anticoagulation at a dose indicated by the Hepcon system. Full heparin reversal is confirmed by a heparin level assay and ACT measurement. Chest tubes are inserted and the sternum is approximated according to procedure and surgeon preference.

After surgery, patients are transferred to the cardiac surgical intensive care unit (CSICU). Standardized protocols for temperature, sedation, pain, glucose, and hemodynamic management are followed. Upon CSICU arrival, active rewarming using a forced-air warming device is performed. Propofol 2 mg/kg/h, ketamine 0.5 mg/kg/h, or dexmedetomidine 0.5-1.5 µg/kg/min are administered and titrated to achieve appropriate sedation. Pain control is achieved with the use of multimodal regimens (I.V. opioids, acetaminophen, and NSAIDS). Within the first 72 hours, blood glucose levels are monitored every 4 hours and I.V. insulin may be used to maintain glucose levels between 100 and 180 mg/dl. Volume status and hemodynamic parameters are continuously monitored throughout the patient CSICU stay by arterial line and PAC. If needed, vasopressors are administered to achieve the following hemodynamic targets: CI >2.2 l/min/m^2, MAP >65 mmHg, and SvO_2 >65%. Cardiac index, pulmonary vascular resistance (PVR), and systemic vascular resistance (SVR) are recorded every 4 hours until test gas suspension. Packed red blood cells (pRBCs) are transfused when Hb <7 g/dl[38]. Urinary output is recorded hourly via urinary catheter. The ICU team may administer furosemide I.V. in presence of oliguria only after optimization of hemodynamic parameters, volume status and assurance of no urinary catheter blockage. No nephrotoxic agents (e.g. I.V. contrast or hydroxyethyl starch products) are administered as recommended by the KDIGO guidelines[1].

Gas delivery

After PAC insertion, patients are randomly allocated to the intervention group (80ppm NO) or the control group (N_2). Using commercially available tanks of N_2 or NO (Airgas Inc, Radnor Township, Pennsylvania) and volumetrically-calibrated flowmeters, pure N_2 (placebo) or 850ppm NO gas in N_2 is mixed with pure O_2 or air. In the NO group, a final concentration of 80ppm NO is obtained. Test gas administration starts at the onset of CPB and lasts for 24 hours. The test gas is administered via the CPB machine into the sweep gas of the CPB oxygenator by regulating the tank as follows:

$$NO\ tank\ flow\ (L/min) = \frac{[sweep\ gas\ flow\ (L/min) \times NO\ concentration\ (80ppm)]}{NO\ tank\ concentration\ (850ppm)}$$

At the end of the CPB, once ventilation is resumed, test gas is delivered via the inspiratory limb of the anesthetic or mechanical ventilator circuit, and thereafter via the mechanical ventilator in the CSICU. The concentration of NO is regulated by using a Medical gas blender. When patients are extubated, they breathe test gas via a facemask or nasal prongs. NO and nitrogen dioxide (NO_2) levels are monitored through in-line NO/NO_2 sensors (Alphasense, Great Notley, Essex, UK). Met-Hb levels and oxygen saturation (SpO_2) are continuously measured using a peripheral pulse co-oximeter (Masimo Rainbow Set™ Technology, Irvine, CA). Schemas illustrating methods of NO and N_2 gas delivery and NO/NO_2 monitoring both during surgery and in the CSICU are reported in Figures 3 and 4.

Abrupt discontinuation of inhaled NO may lead to rebound pulmonary hypertension. This is characterized by worsening oxygenation and increased PAP, resulting in hypoxemia, systemic hypotension, bradycardia, and right ventricular failure[39–41]. For these reasons, after 24 hours, NO is weaned and discontinued while carefully monitoring hemodynamics using the PAC. NO weaning protocols at our institution consists of:

- Decreasing NO to a half dose and maintaining this dose for 5 minutes.
- Further decreasing NO dose to 5ppm and maintaining this dose for 5 minutes.
- Complete discontinuation of NO if no pulmonary hypertension rebound occurs and hemodynamics are stable for 10 minutes after NO gas suspension.

If at any time during the weaning protocol a patient experiences:

- Worsening hypotension (systolic blood pressure, SBP <90 mmHg) requiring the initiation of an I.V. infusion of vasopressor agents or, in patients already receiving vasopressors before NO weaning, a 50% increase of the dose of the vasopressors,

- Worsening hypoxemia requiring an FiO_2 increase of more than 0.2 or a positive end expiratory pressure (PEEP) increase of more than 5 cmH_2O;
- mPAP increase of more than 20% of the value measured before the initiation of the NO weaning, and/or
- A CI decrease below 1.5 L/min/m^2 in the presence of a mPAP over 25 mmHg,

a respiratory therapist (RT) resets the NO to the lowest level at which the patient was stable and notifies the principal investigator (P.I.) and the CSICU attending physician.

Failure of NO-weaning is established when one of the above complications occurs.

Blood and urine collection

To assess the extent of hemolysis and the renal-protective properties of NO administration, plasma samples for plasma free Hb, NO consumption, NO metabolites, and serum creatinine measurements are collected immediately before starting the surgical procedure, 15 minutes after the end of CPB, 24 hours after surgery, and 48 hours after surgery[16,18,42–44]. Additional blood samples for serum creatinine measurements are collected once a day for 7 days following the cardiac procedure or until discharge if the patient is discharged before day 7. Finally, a blood sample for serum creatinine is collected 6 weeks after surgery. Five urine samples are collected for urinary biomarkers of kidney injury (immediately before starting the surgical procedure, 15 minutes after the end of CPB, and at 24 hours, 48 hours and 6 weeks after surgery)[45–47]. After centrifugation, all specimens are coded, snap-frozen with liquid nitrogen, and stored on designated shelves in a -80°C freezer.

Outcomes

Primary outcome

The primary endpoint of this therapeutic trial is to determine whether there is a difference in AKI incidence between the control group (receiving N_2) versus the intervention group (receiving NO). AKI is defined by KDIGO criteria as an abrupt (within 48 hours) reduction in kidney function correlated to an absolute increase in serum creatinine of 0.3 mg/dL or more (≥26.4 µmol/L) or a percentage increase in serum creatinine of 50% or more (1.5-fold from baseline) at any time during the first 7 days after surgery or a urinary output <0.5 ml/Kg/h for >6 hours[48].

Secondary outcomes

Secondary renal outcomes include:

1. AKI severity using the KDIGO stages[48]:
    - Stage 1: Serum creatinine increase ≥26.5 µmol/l (≥0.3 mg/dl) or increase to 1.5-2.0-fold from baseline or urinary output <0.5 ml/kg/h for 6 hours;
    - Stage 2: Serum creatinine increase >2.0-3.0-fold from baseline or urinary output <0.5 ml/kg/h for 12 hours;
    - Stage 3: Serum creatinine increase >3.0-fold from baseline or serum creatinine ≥354 µmol/l (≥4.0 mg/dl) with an acute increase of at least 44 µmol/l (0.5 mg/dl) or urinary output <0.3 ml/kg/h for 24 hours or anuria for 12 hours or a need for renal replacement therapy (RRT).
2. Requirement for RRT following AKI during hospitalization and at 6 weeks, 90 days and 1 year after surgery.
3. Major Adverse Kidney Events (MAKE) at 6 weeks after surgery. MAKE is a composite outcome of death, new RRT, and worsened renal function (defined as a 25% or greater decline in eGFR)[49].

Secondary non-renal outcomes include:

1. Sequential organ Failure Assessment (SOFA) score during ICU stay[50].

2. Prolonged cardiovascular support defined as the need for vasopressors and inotropic agents, a balloon pump, or a ventricular-assist device for more than 48 hours after cardiac surgery.
3. Maximum hourly vasoactive-inotropic score (VIS) for the first 7 days after surgery and duration of vasopressors and inotropic agents support. VIS is calculated as dopamine dose (mcg/kg/min) + dobutamine dose (mcg/kg/min) + 100 x epinephrine dose (mcg/kg/min) + 10 x milrinone dose (mcg/kg/min) + 10,000 x vasopressin dose (units/kg/min) + 100 x norepinephrine dose (mcg/kg/min) + 10 x phenylephrine dose (mcg/kg/min)[51,52].
4. Duration of mechanical ventilation (hours).
5. ICU and hospital length of stay

Exploratory outcomes

1. Severity of AKI using urinary biomarkers of kidney injury[45–47].
2. AKI incidence and severity related to baseline characteristics of enrolled patients such as the presence of eGFR <60 ml/min at baseline, mPAP at baseline, cardiovascular risk factors associated with endothelial dysfunction, the type of surgical procedure, EuroSCORE II[53]) and intraoperative course (i.e. duration of CPB, duration of aortic cross-clamp, levels of plasma free Hb, levels of NO consumption and NO metabolites) that may impact study results.
3. Delirium assessed using the confusion assessment method for the intensive care unit (CAM-ICU) within the first 7 days after surgery or until discharge[54,55].
4. Quality of life at 6 weeks, 90 days, and 1 year after surgery assessed using the Katz Index of Independence in Activities of Daily Living (ADL) and the PROMIS global health questionnaire[56–58].

Safety outcomes

1. Intra-hospital mortality and mortality at 6 weeks, 90 days, and 1 year after surgery.
2. Non-fatal stroke during hospitalization and at 6 weeks after surgery.
3. Perioperative and non-perioperative non-fatal myocardial infarction[59].
4. Postoperative bleeding calculated as the sum of blood loss through thoracic drains from the moment of chest closure over a period of 24 hours.
5. Need for blood transfusions or transfusions with blood products
6. Postoperative infections (e.g., pneumonia, wound infection, endocarditis, central line infection, urinary tract infection, sepsis).
7. Cardiac arrhythmias and other non-cardiac postoperative complications (e.g., hepatobiliary disorders, pneumothorax, pleural effusion, vascular disorders).
8. Patients requiring a decrease of NO delivery due to Met-Hb >5%.

Sample size planning

The sample size needed for this trial is calculated based on the primary endpoint: the reduction of AKI incidence in the NO treated group (intervention group) compared to the N_2 group (control group). In a randomized controlled trial (RCT) conducted at the University of Muenster (Germany), Meersch et al. showed that implementation of a KDIGO CT surgery "bundle" as recommended by the KDIGO guidelines successfully reduced the incidence of CPB-associated AKI from 71.7% to 55.1%[23]. At Massachusetts General Hospital (MGH), the use of the KDIGO guidelines is part of standard care and the CPB-associated AKI rate is similar (55% as observed in a one-year chart review performed by the investigators for surgery requiring CPB >90 minutes [data not shown]). In a prior trial we conducted in China, we found a 22% relative risk reduction in the incidence of AKI and a 42% relative risk reduction of stage 3 CKD at 1 year in the NO treated group[18]. In this study, we estimate a greater reduction in the incidence of AKI (35% relative risk reduction) because we anticipate that an American population with endothelial

dysfunction will benefit more from NO treatment. Thus, in the NO group, the incidence of AKI is expected to decrease from 55% to 35.75%. Using a Fisher's exact test to compare proportions, the sample size needed to detect a difference, assuming a two-sided type I error of 0.05 and a power of 0.8, is 114 patients per group. In order to account for possible dropouts, we have increased our sample size by 10%. The enrollment plan is 250 patients (125 patients in the NO group and 125 patients in the N_2 group).

Recruitment

This study is performed at MGH in Boston, MA. All inpatients and oupatients undergoing a scheduled procedure are screened for eligibility. If a patient is excluded, the reasons are noted on a screening log. To obtain consent from scheduled patients, the details of the study are given during the preoperative visit at the Cardiac Surgery Preoperative Clinic, along with a flyer overviewing the trial protocol and a copy of the consent form. For patients admitted to the hospital, study details are given at least a day prior to their cardiac procedures. On the day of surgery, prior to the initiation of any study procedures, the patient's written consent is obtained by a licensed physician who is also a member of the study staff. Consent is obtained exclusively from the patient, and not from a surrogate. Patients who choose not to participate in this study receive standard care according to the surgical procedure. After consent is obtained, a de-identified code is assigned to the patient and registered on a dedicated enrollment log.

Randomization and treatment allocation

To ensure balance between study groups with respect to the likelihood of receiving NO after surgery, after the insertion of a PAC, the patients are stratified by preoperative mPAP (i.e. mPAP <30 mmHg or mPAP ≥30 mmHg) then allocated into the two study groups. The randomization sequence is created by an independent statistician using Stata Statistical Software: Release 14 (College Station, StataCorp LP, TX, USA). A predetermined block

randomization method is used to ensure equal distribution of participants to treatment arms. A physician with no clinical involvement in providing care for the enrolled patients will conduct the randomization procedure throughout the study. Thus, the randomization is conducted without any influence from clinicians providing care for the patients, or from statisticians or outcome assessors.

Blinding

The NO and N_2 tanks and the gas delivery system in the OR and at the bedside are masked to keep participants, clinicians, and investigators blind to the assignment group. This further ensures allocation concealment since NO and N_2 cannot be distinguished on the basis of appearance. For safety and gas monitoring, the clinician administering the test gas remains unblinded to the treatment. This unblinded clinician is solely responsible for gas tank preparation and test gas delivery and monitoring. Assessors who are blind to the treatment allocation conduct assessment of study outcomes. To maintain the overall quality, legitimacy, and integrity of the clinical trial, unblinding of the test gas may occur only in critical circumstances when the responding physician prescribes initiation of NO. In this circumstance, the P.I. fully documents and explains the reasons for unblinding in a report to the Institutional Review Board (IRB).

Data analysis

All trial outcomes will be evaluated using an intention-to-treat analysis plan. Hypothesis tests will be performed using a two-sided significance level (type I error) of α=0.05. Sensitivity analyses per group of treatment will also be performed and compared to the intention-to-treat analysis results. The two arms will be compared for homogeneity of baseline characteristics, intraoperative course, and postoperative course. Any randomization imbalances or other potential treatment effect modifiers will be examined as covariates in sensitivity analyses

(please see the exploratory outcomes section). To examine baseline differences between the two groups, we will use an unpaired student's t-test for continuous variables with a normal distribution, a Mann-Whitney U test for continuous variables that are not normally distributed, and a Chi-Square test for dichotomous variables. Continuous variables will be described as mean ± standard deviation when normally distributed, median (IQR) when not normally distributed, and count (%) when the variable is categorical. The change in the laboratory markers over time and between the two groups will be tested with a repeated-measures ANOVA. Differences among the groups at each timepoint will be tested using an unpaired student's t-test or a Mann-Whitney U test as appropriate. The incidence of AKI and postoperative adverse events after prolonged CPB will be reported in terms of relative risk with 95% confidence interval (CI) in the treatment versus the control group. The median differences (NO group versus control group) with 95% CI will describe differences in continuous perioperative characteristics and continuous postoperative outcomes.

Interim analysis

An interim analysis by a Data Safety Monitoring Board (DSMB) is planned upon reaching 50% of the study population. The DSMB is comprised of three independent, multidisciplinary experts (online supplementary material: Data and Safety Monitoring Board section). An independent statistician will perform the interim analysis. The statistician will report the results of the data analyzed to the DSMB in a closed session. The DSMB will have access to data collected during the hospital stay and during the follow up visits, including maintenance of patient confidentiality throughout the study. The DSMB may recommend terminating the study if at the interim analysis a significant increase of intra-hospital mortality, mortality at 6 weeks, AKI or need for RRT in either group (N_2 or NO) is detected.

Data management

All data collected for the trial are entered into the MGH Research Electronic Data Capture (REDCap) application[60]. Preoperative, intraoperative, and postoperative data are prospectively collected from the patient's chart until discharge. Follow-up data are collected by interviewing patients at 6 weeks, 90 days and 1 year after surgery.

Data access is restricted and granted by the P.I. only to authorized members of the study team. The P.I. also assigns specific privileges to members of the study team for data import/export. Quality and integrity of the data collected are optimized by using software properties such as logic checks and validation of data fields (i.e. reference range, valid or invalid values). Warnings and error messages alert the co-investigator of missing data or data entries that do not match the requirements set up in the data-entry field. Also, the co-investigators manually perform weekly checks to ensure data consistency. During these periodical checks, all errors detected by the study team related to data collection and data entry are summarized in a data query report. The report is sent to the P.I. following the enrollment of every 5 patients. It is the responsibility of the P.I. to check the original forms for inconsistency, make corrections by modifying the original forms when necessary, and enter a response to the query. Strict confidentiality is maintained by the research team at all times. All forms are and will be kept in a secure, locked cabinet with limited access for at least 5 years after study completion.

Trial management

Study team physicians are responsible for screening all inpatients and outpatients undergoing a scheduled procedure. The number of eligible, consented, enrolled, and randomized patients is recorded in addition to the reasons for non-participation in the trial.

During their hospital stay, patients are closely monitored and all outcomes are recorded. For this reason, no missing endpoints are expected. After discharge, all patients have a scheduled follow-up visit with the cardiac surgeon 6 weeks after surgery. The day before their appointment, the patient is contacted by a member of the study staff to remind them that a member of the

team will collect research data during their visit. This is done by a phone call (or e-mail if unreachable by phone). At the end of the visit with the cardiac surgeon, blood and urine samples are collected for kidney function assessment and a questionnaire regarding medical and surgical complications and quality of life is given. Patients are then called at 90 days and 1 year after surgery to evaluate mid-term and long-term outcomes. To avoid missing follow-ups, a minimum of three calls are attempted by the study team. Calls are made at different times and dates in an attempt to increase the probability of contacting patients. Phone numbers provided by the patient on the screening day are used. If the study team is still unable to contact the patient despite the several phone call attempts, a letter is sent to the home address provided by the patient at their initial screening visit. If all attempts do not provide contact with the patient, a member of the study team may call to the patient's primary care physician or another healthcare provider to obtain information regarding the patient's condition (e.g. deceased). Based on trials previously performed in the Department of Surgery at the MGH, we expect a loss to follow-up of 10%, 15%, and 20% at 6 weeks, 90 days, and 1 year respectively.

Trial risks

Due to the instability of NO, there are risks associated with its use that must be considered. NO reacts slowly with oxygen to form NO_2, which may cause airway inflammation and damage to lung tissues[61]. Moreover, NO oxidizes ferrous Hb to form Met-Hb, which is unable to transport and release oxygen to tissues. However, cyanosis in healthy patients does not appear until Met-Hb levels are 15-20%[62]. The binding of NO to Hb is a rapidly reversible reaction, with a half-life of 15-20 minutes after NO discontinuation. The side effects and adverse events related to NO delivery are well reported in literature. In Table 2 (Summary of Adverse Events from Previous Studies Examining Nitric Oxide), we summarized 8 clinical trials that recorded and reported adverse events associated with NO gas in newborn, pediatric, and adult populations. Based on the present literature and FDA reports[63], the risks of breathing NO at 80ppm for 24 hours are

minimal when Met-Hb levels and NO/NO_2 delivery levels are carefully monitored[64,65]. To improve safety, in the present trial NO is administered and monitored by trained respiratory therapists. The IRB for this study requested monitoring and maintaining levels of NO_2 breathing below 5ppm. We previously tested and found that our delivered circuits do not exceed delivering 1.5ppm of NO_2 when 80ppm of NO gas is delivered at 90% of FiO_2. Met-Hb is continuously monitored by non-invasive co-oximetry[65]. If Met-Hb levels exceed 5% of circulating Hb, the concentration of NO delivered is halved from 80ppm to 40ppm and closely monitored until a reduction occurs. If Met-Hb levels persist above 5%, NO is progressively halved until a reduction below 5% occurs.

Criteria for patient discontinuation

The criteria for patient discontinuation from the study are:

- Voluntary discontinuation by a patient;
- Exiting the protocol for safety reasons based on the judgment of the clinical or research staff. Specific safety reasons include: (1) acute worsening of hypotension defined as a decrease in MAP of >20 mmHg that is not attributable to other causes (such as hypovolemia, hemorrhage or sepsis), but is generally due to worsening of left ventricular failure, (2) sudden worsening of hypoxemia defined as a decrease of SpO_2 to <80% while breathing 100% oxygen and not attributable to other causes (such as pulmonary edema, ARDS, or pulmonary embolism), and (3) an increase in NO_2 levels >5ppm from baseline.

Enrollment to date

As of September 2018, 88 patients have been enrolled in the study. Assuming that we recruit about 8 patients per month, we anticipate completing the recruitment of 250 patients by May 2020.

Ethics and dissemination

The current trial was approved by the International Review Board (IRB) in August 2016. Further amendments of the study protocol have been performed and clinical trials.gov has been updated accordingly. Analysis of the outcomes of the study (primary, secondary and exploratory outcomes) will be performed when the enrollment of 250 patients is complete. These results will be published in a primary paper, which will be directed to a peer-reviewed journal and may be presented as a poster and/or through oral communication at scientific conferences focused on cardiac surgery, nephrology, anesthesia, and critical care medicine. At the end of the trial, upon request, results will be provided to the study participants and/or their family members and then published on clinicaltrtials.gov.

Conclusion

This trial is evaluating whether 24-hour administration of NO gas reduces the incidence of AKI after prolonged CPB in patients with signs and symptoms of endothelial dysfunction. The results obtained by this trial may also represent further progress of the scientific community in evaluating the systemic beneficial properties of exposing plasma to NO gas.

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TABLES

Inclusion criteria:

1. Age ≥ 18 years of age
2. Elective cardiac or aortic surgery with expected CPB > 90 minutes
3. Clinical evidence of endothelial dysfunction assessed by a specifically designed questionnaire (Figure 2)
4. Stable preoperative renal function without evidence of a plasma creatinine increase of ≥ 0.3 mg/dL within 3 months of study entry and without receiving RRT

Exclusion Criteria:

1. eGFR less than 30 ml/min/1.73 m^2
2. Emergent cardiac surgery
3. Life expectancy < 1 year at the time of enrollment
4. mPAP ≥ 40 mmHg and PVR > 4 Wood Units
5. LVEF < 30% by echocardiography obtained within 3 months of enrollment
6. Hemodynamic instability as defined by a SBP <90 mmHg
7. Administration of one or more pRBC transfusion in the week prior to enrollment
8. X-ray contrast infusion less than 48 hours before surgery
9. Evidence of intravascular or extravascular hemolysis from any other origin:
    - Intravascular: Intrinsic RBC defects leading to hemolytic anemia (eg, enzyme deficiencies, hemoglobinopathies, membrane defects). Extrinsic: liver disease, hypersplenism, infections (eg, bartonella, babesia, malaria), treatment with oxidizing exogenous agents (eg, dapsone, nitrites, aniline dyes), exposure to other hemolytic agents (eg, lead, snake and spider bites), lymphocyte leukemia, autoimmune hemolytic disorders
    - Extravascular: Infection (e.g., clostridial sepsis, severe malaria), paroxysmal cold hemoglobinuria, cold agglutinin disease, paroxysmal nocturnal hemoglobinuria, iv infusion of Rho (D) immune globulin, iv infusion of hypotonic solutions

Table 1. Inclusion and exclusion criteria. CPB: Cardio-pulmonary Bypass; RRT: Renal replacement therapy; eGFR: estimated Glomerular Filtration Rate (CKD-EPI formula)[66]; mPAP: Mean pulmonary artery pressure; PVR: Pulmonary vascular resistance; LVEF: Left ventricular ejection fraction; SBP: Systolic blood pressure; pRBC: packed red blood cells

Authors	Number of Patients	Age Group (newborn, pediatric, adult)	NO (ppm)	Time of Exposure	Adverse Events
Van Meurs et al.[67]	210	Premature newborns <34 weeks gestational age (26 ± 2 gestational weeks)	5-10	10-14 hours	NO_2 >5ppm in 2 infants; Met-Hb >8% in 1 infant. No resulting complications were reported
Stork et al.[68]	114	Newborn >34 gestational weeks and <14 days old (39.3 ± 1.8 gestational weeks)	20-80	<14 days	Met-Hb >5% in 11 infants. No resulting complications were reported. A dose of 100ppm NO was given to two infants for 36 and 60 minutes, respectively. NO_2 levels in the second infant elevated to 5.1ppm. NO_2 and Met-Hb levels decreased after NO concentration returned to 80ppm
Roberts et al.[69]	30	Newborns >37 gestational weeks (39.8 ± 1.5 gestational weeks)	80	<8.5 days	Met-Hb elevated to 18.2% in 1 infant. The later clinical course was uneventful
James et al.[70]	101	Pediatric (1-43 months old)	20	1-3 hours	None related to NO delivery
Cueto et al.[71]	40	Pediatric (15 days old - 17 years old)	4-30	hours to days	Rebound effects of NO withdrawal reported in 2 patients
Lei et al.[18]	117	Adult >18 years old (48.7 ± 9.5 years old)	80	24 hours	None related to NO delivery
Rossaint et al.[72]	9	Adult (17-46 years old)	5-20	3-53 days	None related to NO delivery
Taylor et al.[73]	192	Adult >18 years old (50 ± 17 years old)	5	<28 days	66 reported infections in the NO group and 41 in the control group. Infections deemed unrelated to NO gas administration. No further complications were reported

Table 2. Summary of prior selected studies that reported adverse events related to nitric oxide administration. Data of age are presented as mean ± SD or range.

FIGURES

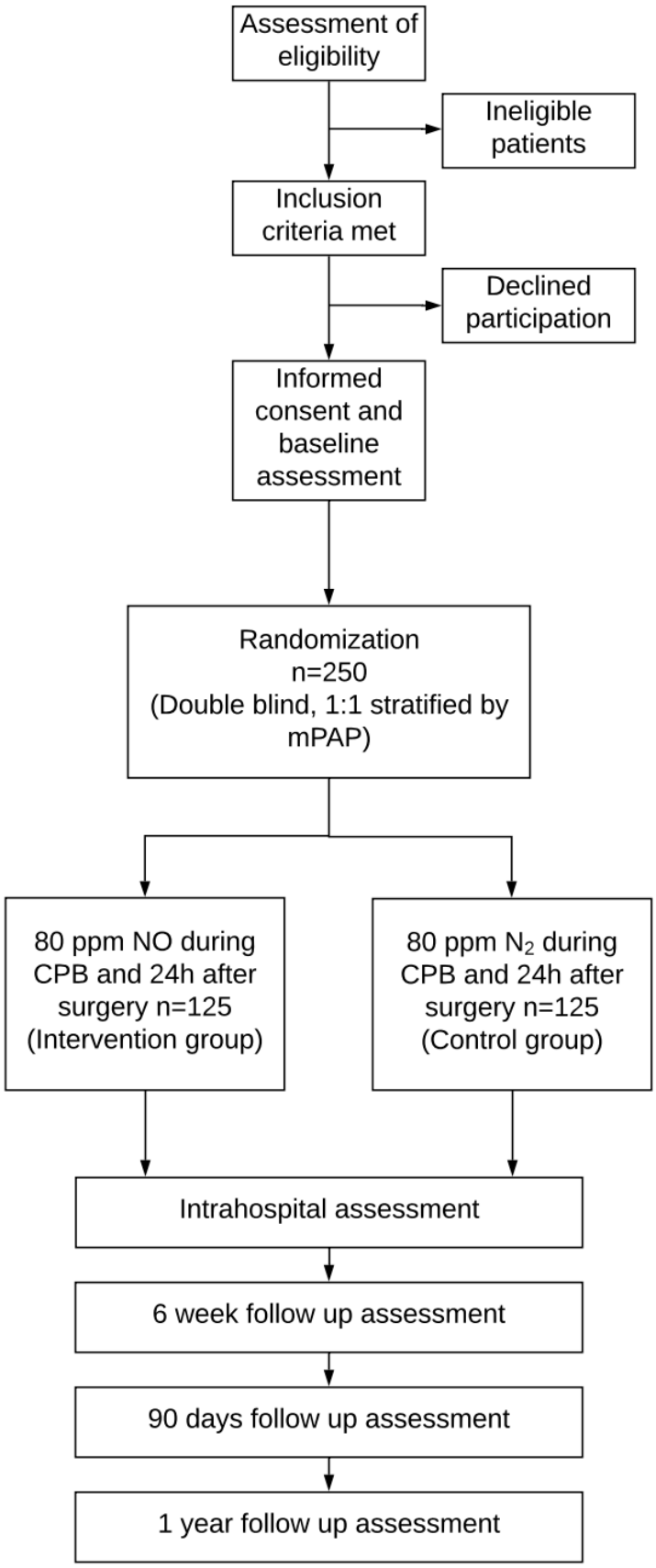
Figure 1. **Study design.** NO: Nitric Oxide; CPB: cardio-pulmonary bypass; N_2: Nitrogen; mPAP: mean pulmonary artery pressure. After placement of pulmonary artery catheter, to ensure balance between study groups with respect to the likelihood of receiving NO after surgery, patients are randomized based on mPAP measured by the pulmonary artery catheter placed on the day of surgery (mPAP <30mmHg and mPAP between 30 mmHg and 39 mmHg).

Figure 2. Screening questionnaire to detect endothelial dysfunction. The questionnaire above aims to systematically detect endothelial dysfunction in patients undergoing a cardiac surgical procedure. If "yes" is answered to at least 1 of the above questions, the patient can be considered to have endothelial dysfunction and he/she may be enrolled in the study. PTCA: Percutaneous transluminal coronary angioplasty; IDDM: Insulin dependent diabetes mellitus; NIDDM: Non-insulin dependent diabetes mellitus; LDL: Low-density lipoprotein; BMI: Body mass index; SBP: Systolic blood pressure;

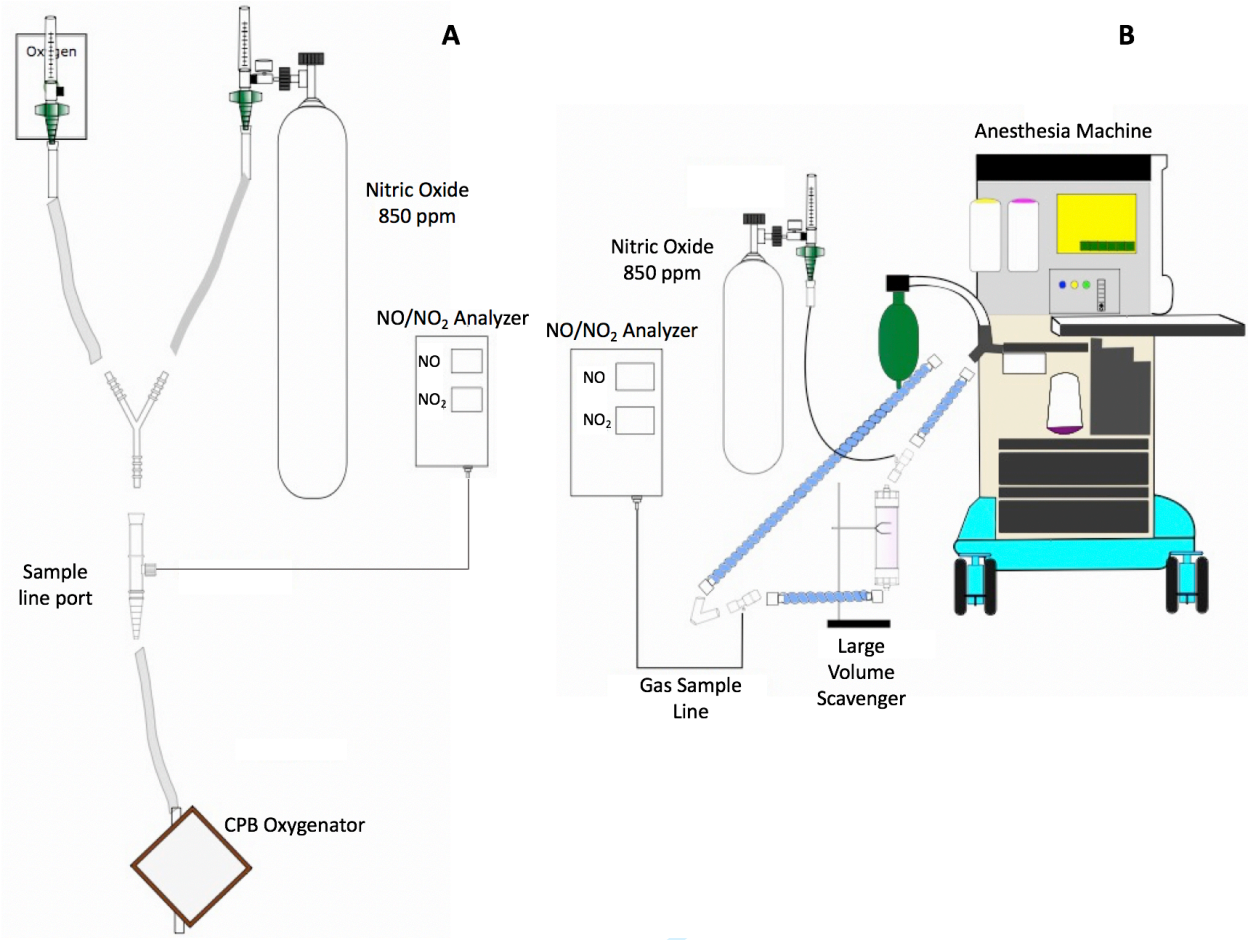
Figure 3: Schema of the NO delivery systems in the operating room. A. Figure illustrating how the NO or N_2 is delivered into the CPB oxygenator. Tanks of pure N_2 are used in the control group and tanks of 850ppm NO in N_2 are used in the intervention group. A "Y" adaptor is inserted into the sweep gas input line leading to the oxygenator. This allows for the mixing of the test gas with the sweep gas (O2 + medical air). This mixture is periodically monitored with an NO/NO_2 analyzer directly before entering the oxygenator. **B.** Figure illustrating how the NO or N_2 is delivered into the anesthesia ventilator once ventilation has been resumed. The test gas is delivered by placing a "Y" adaptor into the inspiratory limb of the circuit. The mixture is scrubbed of NO_2 by a large volume scavenger containing calcium hydroxide and is periodically analyzed with a NO/NO_2 analyzer before being inhaled by the patient.

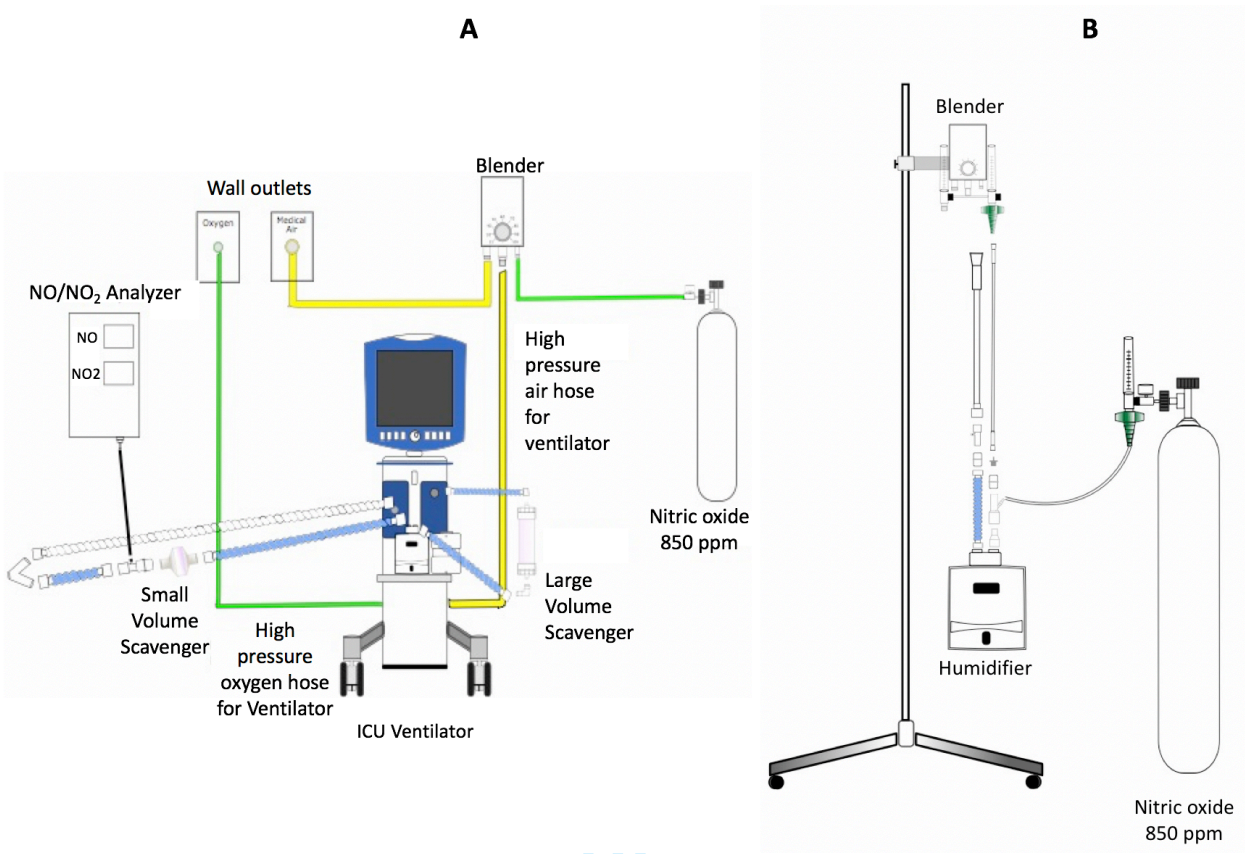
Figure 4: Schema of the NO delivery systems in the cardiac surgical intensive care unit.

A. Figure illustrating how the NO or N_2 is delivered through the mechanical ventilator at bedside in the ICU. Tanks of pure N_2 are used in the control group and tanks of 850ppm NO in N_2 are used in the intervention group. Test gas is blended with medical air and enters the air inlet of the ventilator. The high pressure O_2 hose is directly connected to the ventilator. If there is any change of FiO_2, the amount of NO/N_2 delivered is regulated by the RT by adjusting the blender setting and the ventilator FiO_2 setting insuring that the patient is still receiving the target concentration of 80ppm NO. The mixture obtained is then scrubbed of NO_2 through a large volume scavenger and a small volume scavenger placed in series on the inspiratory limb of the circuit. The final amount of NO and NO_2 delivered is periodically analyzed with a NO/NO_2 analyzer directly before the mixture is inhaled by the patient. **B.** Figure illustrating how the NO or N_2 is delivered into the High Flow Nasal Cannula device. The test gas is delivered to the system by placing "Y" adaptor before the humidifier. A commercially available blender mixes O2 and medical air and is regulated by the RT to reach the target FiO_2. The flow of NO_2 or N_2 is titrated to reach the desired concentration 80ppm NO or placebo. This mixture is then humidified and heated to a temperature of 34°C.



CABG and/or valve repair/replacement and age > 40 year old for males and > 50 year old for females and 1 out of 8 of the following criteria:	
Previous coronary artery bypass graft or PTCA (+ stent)	Yes___ No___
History or presence of intermittent claudication, critical limb ischemia, or peripheral vascular disease with the exception of vasculitis.	Yes___ No___
History of transient ischemic attack and/or ischemic stroke	Yes___ No___
Diagnosis of diabetes (IDDM or NIDDM) requiring oral hypoglycemic agents or insulin	Yes___ No___
Hypercholesterolemia (total cholesterol > 200 mg/dl or LDL > 160 mg/dl) treated with statins, ion-exchange resins or other oral agents	Yes___ No___
BMI > 40	Yes___ No___
Hypertension (SBP 140 ≥ mmHg) treated with antihypertensive drugs	Yes___ No___
Active smoking ≥ 10 pack - years	Yes___ No___





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Primary Registry and Trial Identifying Number	**ClinicalTrials: NCT02836899**
Date of Registration	July 19 2016
Secondary Identifying Numbers	IRB ID#: 2016 P001629
Source(s) of Monetary Support	National Heart, Lung, and Blood Institute (NHLBI) (Award Reference Number K23 HL128882-01A1)
Primary Sponsor	Department of Anesthesiology, Critical care and Pain Medicine Massachusetts General Hospital Boston, MA 02114
Contact name	Lorenzo Berra, MD lberra@mgh.harvard.edu 617-724-2000 Department of Anesthesiology, Critical care and Pain Medicine Massachusetts General Hospital

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Public Title	Effect of Nitric Oxide in Cardiac Surgery Patients With Endothelial Dysfunction
Scientific Title	Prevention of Acute Kidney Injury by Nitric Oxide in Prolonged Cardiopulmonary Bypass: A Double-Blind Controlled Randomized Trial in Cardiac Surgical Subjects with Endothelial Dysfunction
Countries of Recruitment	United States
Health Condition(s) or Problem(s) Studied	Cardiopulmonary bypass associated-Acute Kidney Injury

Intervention(s)	Trial arm 1: Nitric oxide (NO) Group (intervention arm). NO gas 80 parts-per-million is administered via the oxygenator during CPB, then by inhalation when mechanical ventilation is resumed. Once the subject is extubated, NO gas is delivered by high flow nasal cannula.The treatment begins at the onset of the cardiopulmonary bypass and lasts 24h. Trial arm 2: Nitrogen (N_2) Group (Control arm). N_2 gas is administered via the oxygenator during CPB, then by inhalation when mechanical ventilation is resumed. Once the subject is extubated, N_2 gas is delivered by high flow nasal cannula. The N_2 administration begins at the onset of the cardiopulmonary bypass and lasts 24h.
Trial Type	Interventional Allocation: Randomized Intervention model: Parallel assignment Blinding: Clinicians blinded to intervention, participants blinded to intervention, study

	investigators blinded to intervention, primary outcome assessor blinded to intervention. For safety and gas monitoring, only the clinician administering the test gas remains unblind to the treatment. This clinician is not part of the anesthesia, ICU, or surgical physician team delivering care. Assignment: Parallel Primary purpose: Prevention
Date of First Enrollment	June, 2017
Target Sample Size	250 patients
Recruitment Status	Recruiting
Key Enrollment Criteria	Inclusion criteria: Age ≥ 18 years of age; Elective cardiac or aortic surgery with expected CPB > 90 minutes; Clinical evidence of endothelial dysfunction assessed by a specifically designed questionnaire; Stable preoperative renal function without evidence of a plasma creatinine increase of ≥ 0.3 mg/dL within 3 months of study entry and without receiving RRT. Key exclusion criteria:

	estimated glomerular filtration rate (eGFR) <30 ml/min/1.73m^2, mPAP ≥40 mmHg and intravenous (I.V.) contrast infusion within 48 hours before surgery.
Primary Outcome(s)	Outcome name: Incidence of Acute Kidney Injury (AKI) Method of measurement: KDIGO criteria Time points of interest: 7 days after surgery
Key Secondary Outcomes	Outcome name: AKI severity Method of measurement: KDIGO stages Time points of interest: 7 days after cardiac surgery Outcome name: Renal Replacement Therapy Method of measurement: Medical record review Time points of interest: Up to 1 year. Outcome name: Major Adverse Kidney Events (MAKE) Method of measurement: Medical record

review

Time points of interest: 6 weeks after cardiac surgery.

Outcome name: Organ dysfunction (SOFA score)

Method of measurement: Medical record review

Time points of interest: ICU stay (up to seven days)

Outcome name: Prolonged cardiovascular support

Method of measurement: Medical record review

Time points of interest: 48 hours after cardiac surgery

Outcome name: duration of mechanical ventilation

Method of measurement: Medical record review

Time points of interest: up to 6 weeks

Outcome name: Intensive care unit length of stay (ICU-LOS)

Method of measurement: Medical record review

Time points of interest: up to 6 weeks

Outcome name: Hospital length of stay (LOS)

Method of measurement: Medical record review

Time points of interest: up to 1 year

Data and Safety Monitoring Board:

DSMB Chair:

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Responsibilities include: reviewing and evaluating the trial data to ensure participant safety, trial conduct, progress, and efficacy, and making recommendations regarding the continuation, or termination of the trial.

Partners HealthCare System Research Consent Form

Subject Identification

General Template
Version Date: August 2016

Protocol Title:

Principal Investigator:

Site Principal Investigator:

Description of Subject Population:

INSTRUCTIONS: Many sections of this document include brief instructions to provide the user with a general overview of information required in the section. The instructions are shaded so that you can tell the difference between the instructions and required information. Some sections are password protected and cannot be edited. Detailed instructions for preparing consent forms are available at: **https://partnershealthcare.sharepoint.com/sites/phrmApply/aieipa/irb/Pages/Research-Consent-Form-Templates.aspx.**

Please delete all shaded instruction boxes prior to submitting this form to the Partners Human Research Committee (PHRC) for review. To delete, select a shaded box and click the cut button on the Word toolbar.

About this consent form

Please read this form carefully. It tells you important information about a research study. A member of our research team will also talk to you about taking part in this research study. People who agree to take part in research studies are called "subjects." This term will be used throughout this consent form.

Partners HealthCare System is made up of Partners hospitals, health care providers, and researchers. In the rest of this consent form, we refer to the Partners system simply as "Partners."

If you have any questions about the research or about this form, please ask us. Taking part in this research study is up to you. If you decide to take part in this research study, you must sign this form to show that you want to take part. We will give you a signed copy of this form to keep.

Partners HealthCare System
Research Consent Form

Subject Identification

General Template
Version Date: August 2016

INSTRUCTIONS: Include the following paragraph **only** if some or all of the adult subjects are incapable of providing consent and permission for their participation will be obtained from their authorized representative. Delete the following paragraph when all subjects are adults capable of providing consent.

Some of the people who are eligible to take part in this study may not be able to give consent to take part because of their medical condition. Instead we will ask the person's authorized representative to give consent. Throughout the consent form, "you" always refers to the person who takes part in the study.

INSTRUCTIONS: Include the following paragraph **only** when some of the subjects are minors (less than 18 years of age) and permission for their participation will be obtained from their parent(s)/guardian. Delete the following paragraph when all subjects are adults.
Note: For studies that are limited to minors, use the Consent Form for Parents, and if minors are less than 14, you must also prepare a Youth Assent Form.

Some of the people who are eligible to take part in this study may not be able to give consent because they are less than 18 years of age (a minor). Instead we will ask their parent(s) to give permission for them to take part in the study and will ask them to agree (give their assent) to take part. Throughout the consent form, "you" always refers to the person who takes part in the study.

INSTRUCTIONS: Include the following paragraph if the study will be registered on clinicaltrials.gov to meet FDAAA clinical trials registration requirements. This paragraph must be included even if the sponsor is the responsible party for clinical trials registration. This paragraph is not required when registering only to meet journal requirements.

A description of this clinical trial will be available on *http://www.ClinicalTrials.gov,* as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

Why is this research study being done?

INSTRUCTIONS: The following information is provided to help you prepare this section of your consent form. Include the following information, when applicable, in this section:

- Purpose of the research, e.g., "We are doing this research to…"
- Information about the drug/device, including FDA status, e.g., "The drug/device is/is not

approved by the U.S. Food and Drug Administration (FDA) to treat...."

- Reason for asking these individuals to take part, e.g., "We are asking you to take part in this research study because you…"
- Expected enrollment, e.g., "About 100 people will take part in this research study. About 20 subjects will take part at Brigham and Women's Hospital (BWH)."
- Name of corporate sponsor/funding agency/foundation, e.g., "[Sponsor/Funding Agency/Foundation] is paying for this research to be done."

How long will I take part in this research study?

INSTRUCTIONS: The following information is provided to help you prepare this section of your consent form. Include the following information in this section:

- Expected time commitment to complete the study, e.g., "It will take you about 14 months to complete this research study. During this time, we will ask you to make 14 study visits to [BWH/MGH]."

What will happen in this research study?

INSTRUCTIONS: The following information is provided to help you prepare this section of your consent form. Include the following information, when applicable, in this section:

- Description of the study visits and procedures the research participants will undergo (whenever possible, organize the information chronologically by study visit, and use headings for visits and bullets to list procedures; indicate how long visits will take)
- Information about the study design, e.g., randomization, placebo, blinding
- Special requirements, e.g., stopping current medications, fasting before tests
- Off-site testing, e.g., MRI center in Charlestown
- Partners Alert System, include statement, **"**Partners has an electronic system that lets your study doctors know if you are admitted to a Partners Hospital, or if you visit a Partners Hospital Emergency Department. We want to make sure the study doctors know about any possible problems or side effects you experience while you are taking part in the study."
- Reasons for and procedures for early withdrawal from the study, e.g., tapering medications, final study visit
- Sending data/specimens to research collaborators outside Partners
- Storage of data/specimens for future use, include the following:

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Storing Samples and Health Information at [BWH/MGH] for Future Use
We would like to store some of your samples and health information for future research related to [general disease areas]. We will label your samples and health information with a code instead of your name. The key to the code connects your name to your samples and health information. The study doctor will keep the key to the code in a [password protected computer/locked file].

Do you agree to let us store your samples and health information for future research related to [general disease area]?

☐ Yes ☐ No Initials_____

If later you change your mind and want your samples destroyed, contact the study doctor.

- Study information that will be included in the electronic medical record: In most studies, some information from the research will become part of the subject's electronic medical record. Include one of the statements below. The IRB will review your selection of statement, and if it agrees, will approve the use of the statement for the study.

Study Information Included in Your Electronic Medical Record
[Statement 1: Use this statement for most studies.]
A notation that you are taking part in this research study may be made in your electronic medical record. Information from the research that relates to your general medical care may be included in the record (for example, list of allergies, results of standard blood tests done at the hospital labs).
[Statement 2: Use this statement if you consider the study topic to be highly sensitive (for example, studies of sexual practice; sexual victimization; illegal behaviors; alcohol, drugs or other addictive products; or stigmatizing illnesses) such that the study title should not appear in the subject's medical record.]
A notation that you are taking part in this research study may be made in your electronic medical record. **For this study, only a study number, and NOT the title of the study, will be in your record: for example Study #123.** Information from the research that relates to your general medical care may be included in the record (for example: list of allergies, results of standard blood tests done at the hospital labs).

Please ask your study doctor if you have any questions about what information will be included in your electronic medical record.

What are the risks and possible discomforts from being in this research study?

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INSTRUCTIONS: The following information is provided to help you prepare this section of your consent form. The information in this section should be limited to the risks and discomforts related to the procedures done for research purposes, and should not include those related to a research participant's routine medical care. Be careful not to minimize risks or discomforts. Include the following information in this section:

- Reasonably foreseeable physical, psychological, economic, legal, or social risks, or discomforts that may result from study procedures (drugs, devices, tests), or from a breach in confidentiality
- Unforeseeable risks that may result from study drugs, devices, procedures, e.g., "There may be other risks that are currently unknown."

What are the possible benefits from being in this research study?

INSTRUCTIONS: The following information is provided to help you prepare this section of your consent form. Do not include compensation as a benefit. Include the following information in this section:

- Reasonably expected benefits to the participant (if any)
- Reasonably expected benefits to future patients with the disease/condition being studied.

What other treatments or procedures are available for my condition?

INSTRUCTIONS: The following information is provided to help you prepare this section of your consent form. Include the following information in this section:

- Appropriate alternative procedures or courses of treatment, if any, that might be advantageous to the participant. Be specific; when mentioning alternative drugs used to treat the medical condition being studied, provide the name of 3-5 alternative drugs. For example: "You do not have to take part in this study to be treated for [medical condition being studied]. Other treatments or procedures that are available to treat [medical condition being studied] include:
  - [list alternatives]"
- Palliative care or no treatment, when appropriate

Note: This section may not be relevant for all studies. You may delete this section heading if the study involves healthy volunteers and/or is designed to study human physiology. This

section should be included when the research is designed to test the safety and/or effectiveness of a procedure or course of treatment, or if the tests or evaluations are available outside the study.

Can I still get medical care within Partners if I don't take part in this research study, or if I stop taking part?

Yes. Your decision won't change the medical care you get within Partners now or in the future. There will be no penalty, and you won't lose any benefits you receive now or have a right to receive.

Taking part in this research study is up to you. You can decide not to take part. If you decide to take part now, you can change your mind and drop out later. We will tell you if we learn new information that could make you change your mind about taking part in this research study.

What should I do if I want to stop taking part in the study?

If you take part in this research study, and want to drop out, you should tell us. We will make sure that you stop the study safely. We will also talk to you about follow-up care, if needed.

Also, it is possible that we will have to ask you to drop out of the study before you finish it. If this happens, we will tell you why. We will also help arrange other care for you, if needed.

Will I be paid to take part in this research study?

INSTRUCTIONS: The following information is provided to help you prepare this section of your consent form. Include the following information in this section:

- Money or other forms of compensation or reimbursement, e.g., gift certificate, meal voucher, parking voucher, and travel expenses
- Include how the amount of compensation is calculated if the participant does not complete the entire study for any reason, e.g., "If you do not complete the study, we will pay you $25 for each visit you complete."

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Note: This section may not be relevant for all studies. You may delete this section heading if subjects will not be paid or will not receive other forms of compensation for participation. This section must be included when research participants will be paid or will receive some other form of compensation for taking part in the research, such as reimbursement of travel or parking expenses.

What will I have to pay for if I take part in this research study?

INSTRUCTIONS: The following information is provided to help you prepare this section of your consent form. Include the following information in this section:

- Costs that are paid for by study funds, e.g., "Study funds will pay for the study drug and the MRI that is done only for research."
- Any additional costs to the participant that may result from participation in the research, including costs associated with routine care billed to health insurers

A common misconception amongst patients is that if they participate in a research study all of the costs of their care will be covered by the research sponsor. Although this is true in a few cases, the hospital is entitled to and generally will bill a subject's health insurer for, among other things, routine care that the subject would have received had they not participated in the study. Although the amounts vary by insurer, a research subject is likely to be responsible for co-pays and deductibles associated with this routine care. It is important to make sure that patients who volunteer to participate in your research study understand their potential financial responsibility. If these or other costs billable to insurance or billable to the subject directly can be identified in advance (such as through a Medicare Coverage Analysis billing grid), it is a good idea to give the subject notice of specific items or services that may result in significant financial responsibility for the subject. If specific amounts cannot be identified in advance, you should make sure that the subject understands that they might incur some financial responsibility as a result of their participation.

At a minimum, you must include the following language in the consent form:

"Study funds will pay for certain study-related items and services. We may bill your health insurer for, among other things, routine items and services you would have received even if you did not take part in the research. You will be responsible for payment of any deductibles and co-payments required by your insurer for this routine care or other billed care. If you have any questions about costs to you that may result

from taking part in the research, please speak with the study doctors and study staff. If necessary, we will arrange for you to speak with someone in Patient Financial Services about these costs."

Note: You may add further language to describe specific items/services/amounts that will be the subject's responsibility, but you may not delete any portion of the standard language.

What happens if I am injured as a result of taking part in this research study?

INSTRUCTIONS: Include the following paragraph if this study is being conducted at BWH/F, MGH or NSMC.

We will offer you the care needed to treat any injury that directly results from taking part in this research study. We reserve the right to bill your insurance company or other third parties, if appropriate, for the care you get for the injury. We will try to have these costs paid for, but you may be responsible for some of them. For example, if the care is billed to your insurer, you will be responsible for payment of any deductibles and co-payments required by your insurer.

INSTRUCTIONS: Include the following paragraphs if this study is being conducted at McLean Hospital.

If you are injured as a direct result of taking part in this research study, we will assist you in obtaining the medical care needed to treat the injury. This means arranging for (but not paying for) transportation to an acute care center for treatment of the injury. McLean Hospital is a psychiatric care facility and does not provide general health care services.

The care provider may bill your insurance company or other third parties, if appropriate, for the care you get for the injury. We will try to have these costs paid for, but you may be responsible for some of them. For example, if the care is billed to your insurer, you will be responsible for payment of any deductibles and co-payments required by your insurer.

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INSTRUCTIONS: The sponsor may request to include a statement about the injury coverage the sponsor will offer. When the sponsor requests to include such a statement, the statement may be entered below, after the institution's commitment to provide care for the injury. For example: "In this study, [Sponsor] will pay for medical treatment for any injury that is not paid for by your health insurer if the injury is a direct result of your taking part in the study. [Sponsor] has no plans to offer you any other payments or other type of compensation."

Injuries sometimes happen in research even when no one is at fault. There are no plans to pay you or give you other compensation for an injury, should one occur. However, you are not giving up any of your legal rights by signing this form.

If you think you have been injured or have experienced a medical problem as a result of taking part in this research study, tell the person in charge of this study as soon as possible. The researcher's name and phone number are listed in the next section of this consent form.

If I have questions or concerns about this research study, whom can I call?

You can call us with your questions or concerns. Our telephone numbers are listed below. Ask questions as often as you want.

[Insert name and academic degrees] is the person in charge of this research study. You can call him/her at [Insert phone number] [insert when person is available M-F 9-5 or 24/7]. You can also call [Insert name(s)] at [Insert phone number(s)] [insert when each person is available M-F 9-5 or 24/7] with questions about this research study.

If you have questions about the scheduling of appointments or study visits, call [Insert name(s)] at [Insert phone number(s)].

If you want to speak with someone **not** directly involved in this research study, please contact the Partners Human Research Committee office. You can call them at 857-282-1900.

You can talk to them about:

- Your rights as a research subject
- Your concerns about the research
- A complaint about the research

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Also, if you feel pressured to take part in this research study, or to continue with it, they want to know and can help.

If I take part in this research study, how will you protect my privacy?

During this research, identifiable information about your health will be collected. In the rest of this section, we refer to this information simply as "health information." In general, under federal law, health information is private. However, there are exceptions to this rule, and you should know who may be able to see, use, and share your health information for research and why they may need to do so.

In this study, we may collect health information about you from:

- Past, present, and future medical records
- Research procedures, including research office visits, tests, interviews, and questionnaires

Who may see, use, and share your identifiable health information and why they may need to do so:

- Partners research staff involved in this study
- The sponsor(s) of this study, and the people or groups it hires to help perform this research
- Other researchers and medical centers that are part of this study and their ethics boards
- A group that oversees the data (study information) and safety of this research
- Non-research staff within Partners who need this information to do their jobs (such as for treatment, payment (billing), or health care operations)
- The Partners ethics board that oversees the research and the Partners research quality improvement programs.
- People from organizations that provide independent accreditation and oversight of hospitals and research
- People or groups that we hire to do work for us, such as data storage companies, insurers, and lawyers
- Federal and state agencies (such as the Food and Drug Administration, the Department of Health and Human Services, the National Institutes of Health, and other US or foreign government bodies that oversee or review research)

- Public health and safety authorities (for example, if we learn information that could mean harm to you or others, we may need to report this, as required by law)
- Other:

Some people or groups who get your health information might not have to follow the same privacy rules that we follow and might use or share your health information without your permission in ways that are not described in this form. For example, we understand that the sponsor of this study may use your health information to perform additional research on various products or conditions, to obtain regulatory approval of its products, to propose new products, and to oversee and improve its products' performance. We share your health information only when we must, and we ask anyone who receives it from us to take measures to protect your privacy. The sponsor has agreed that it will not contact you without your permission and will not use or share your information for any mailing or marketing list. However, once your information is shared outside Partners, we cannot control all the ways that others use or share it and cannot promise that it will remain private.

Because research is an ongoing process, we cannot give you an exact date when we will either destroy or stop using or sharing your health information.

The results of this research study may be published in a medical book or journal, or used to teach others. However, your name or other identifying information **will not** be used for these purposes without your specific permission.

Your Privacy Rights

You have the right **not** to sign this form that allows us to use and share your health information for research; however, if you don't sign it, you can't take part in this research study.

You have the right to withdraw your permission for us to use or share your health information for this research study. If you want to withdraw your permission, you must notify the person in charge of this research study in writing. Once permission is withdrawn, you cannot continue to take part in the study.

If you withdraw your permission, we will not be able to take back information that has already been used or shared with others.

You have the right to see and get a copy of your health information that is used or shared for treatment or for payment. To ask for this information, please contact the person in charge of this research study. You may only get such information after the research is finished.

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Informed Consent and Authorization

Statement of Person Giving Informed Consent and Authorization

- I have read this consent form.
- This research study has been explained to me, including risks and possible benefits (if any), other possible treatments or procedures, and other important things about the study.
- I have had the opportunity to ask questions.
- I understand the information given to me.

GENERAL INSTRUCTIONS: Include signature line(s) as appropriate to the subject population and consent process described in the protocol documents. Delete those signature lines that are not applicable. Note: Time is highly recommended when study procedures will be performed on the same day as informed consent is documented. Otherwise time is optional.

INSTRUCTIONS: Include the following signature line when informed consent and authorization for participation of some or all subjects will be obtained directly from the subjects.

Signature of Subject:

I give my consent to take part in this research study and agree to allow my health information to be used and shared as described above.

Subject	Date	Time (optional)

INSTRUCTIONS: Include the following signature line when informed consent and authorization for participation of some or all child subjects will be obtained from parents/guardian.

Signature of Parent(s)/Guardian for Child:

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I give my consent for my child to take part in this research study and agree to allow his/her health information to be used and shared as described above.

Parent(s)/Guardian for Child | Date | Time (optional)

INSTRUCTIONS: Include the following signature line when informed consent and authorization for participation of some or all adult subjects will be obtained from a guardian, health care proxy, durable power of attorney, or family member/next-of-kin.

Signature of Guardian or Authorized Representative for Adult:

I give my consent for the person I am authorized to represent to take part in this research study and agree to allow his/her health information to be used and shared as described above.

Print Name (check applicable box below)

- ☐ Court-appointed Guardian
- ☐ Health Care Proxy
- ☐ Durable Power of Attorney
- ☐ Family Member/Next-of-Kin

Signature | Date | Time (optional)

INSTRUCTIONS: Include a line for relationship to adult subject when informed consent and authorization for participation of some or all adult subjects will be obtained from a family member/next-of-kin.

Relationship to Subject:_____

INSTRUCTIONS: Include this section when assent of children ages 14-17 or of decisionally-impaired adult subjects will be obtained. Do not include this section for assent of children ages

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7-13. For assent of children ages 7-13, use the separate Youth Assent Form.

Assent

Statement of Person Giving Assent

- This research study has been explained to me, including risks and possible benefits (if any), other possible treatments or procedures, and other important things about the study.
- I have had the opportunity to ask questions, and my questions have been answered.

INSTRUCTIONS: Include signature line(s) for children ages 14-17 or decisionally-impaired adult subjects as appropriate to the subject population and assent process described in the protocol documents. Delete those signature lines that are not applicable. When assent of subjects will be obtained, always include at least one of the following signature lines.

Signature of Child:

I agree to take part in this research study and agree to allow my health information to be used and shared as described above.

_____ _____ _____

Child, Ages 14-17 Date Time (optional)

Signature of Adult:

I agree to take part in this research study and agree to allow my health information to be used and shared as described above.

_____ _____ _____

Adult Date Time (optional)

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Signature of Study Doctor or Person Obtaining Consent:

Statement of Study Doctor or Person Obtaining Consent

- I have explained the research to the study subject.
- I have answered all questions about this research study to the best of my ability.

Study Doctor or Person Obtaining Consent	Date	Time (optional)

INSTRUCTIONS: The PHRC does not routinely require a subject advocate be involved in the consent process; therefore, delete this section unless the sponsor requires a subject advocate, or you plan to use a subject advocate. Should the PHRC require a subject advocate, they will instruct you to add the following signature line to the consent form.

Subject Advocate

In certain situations, the Partners Human Research Committee (PHRC) will require that a subject advocate also be involved in the consent process. The subject advocate is a person who looks out for the interests of the study subject. This person is not directly involved in carrying out the research. By signing and dating below, the subject advocate represents (or "says") that the subject has given meaningful consent to take part in the research study.

Statement of Subject Advocate

I represent that the subject or authorized individual signing above has given meaningful consent.

Subject Advocate (when required)	Date	Time (optional)

INSTRUCTIONS: Include the following signature line when you anticipate using the "short form" consent process to obtain and document informed consent of subjects who do not speak English. For more information, refer to **https://partnershealthcare-public.sharepoint.com/ClinicalResearch/Non-English_Speaking_Subjects.pdf.**

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Consent of Non-English Speaking Subjects Using the "Short Form" in the Subject's Spoken Language

Statement of Hospital Medical Interpreter

As someone who understands both English and the language spoken by the subject, I interpreted, in the subject's language, the researcher's presentation of the English consent form. The subject was given the opportunity to ask questions.

Hospital Medical Interpreter	Date	Time (optional)

OR

Statement of Other Individual (Non-Interpreter)

As someone who understands both English and the language spoken by the subject, I represent that the English version of the consent form was presented orally to the subject in the subject's own language, and that the subject was given the opportunity to ask questions.

Name	Date	Time (optional)

INSTRUCTIONS: Include the following signature line when you anticipate enrolling subjects who cannot read or write in any language or subjects who are physically unable to talk or write.

Witness to Consent of Subjects Who Cannot Read or Write or are Physically Unable to Talk or Write

Statement of Witness

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I represent that the consent form was presented orally to the subject in the subject's own language, that the subject was given the opportunity to ask questions, and that the subject has indicated his/her consent and authorization for participation by (check one box as applicable):

☐ Making his/her mark above

☐ Other means _____
(fill in above)

_____ _____ _____
Witness Date Time (optional)

Consent Form Version:

INSTRUCTIONS: The unlocked area below is provided to help the study site manage consent documents and versions. You may use this unlocked area to type in the file name and location (path name) of the consent document. Alternatively, you may choose to use one of several tools available in Word that automatically adds file name and location and/or date created to the document, as specified by the user. **NOTE: THE USE OF THIS UNLOCKED AREA IS OPTIONAL.**
For example:
Consent Form Created on: 10/18/14 12:57 PM
Consent Form Path/File Name: sfa\RAHRC\PHSResearchConsentForm

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

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

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

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

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

Chan A-W, Tetzlaff JM, 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
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	3
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	See note 1
Protocol version	#3	Date and version identifier	2
Funding	#4	Sources and types of financial, material, and other support	2
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	See note 2
Roles and responsibilities:	#5b	Name and contact information for the trial sponsor	See note 3

sponsor contact information			
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	See note 4
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)	See note 5
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	7,8
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	8
Objectives	#7	Specific objectives or hypotheses	8,9
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)	8
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, 19, 20
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)	9, 10, 35, 36
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10-16

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)	25
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	23-24
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	23
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	16-19
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, 24
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	19, 20
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	19, 20
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	20, 21
Allocation concealment	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed	20,21

mechanism		envelopes), describing any steps to conceal the sequence until interventions are assigned	
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	20, 21
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	21
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	21
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	22-24
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	22-24
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	22-24
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	21,22
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	21,22
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)	21,22

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	22 and suppl 16
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	22
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	21, 24, 25
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	22, 23
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	21, 26
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)	26
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	20
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	See note 6
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	22-24
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	2

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	22, 23
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	See note 7
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	26
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	See note 8
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	See note 9
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	16

Author notes

1. Suppl material: 9-15
2. Page 1, 3 and suppl material page 1-9
3. Suppl material: 9
4. N/A No role of the study sponsors and funders.
5. Suppl material: 16
6. To participate in this trial, one consent is collected. As indicated in question #33: We plan for collection, laboratory evaluation, and storage of biological specimens analysis in the current trial for possible ancillary studies.

7. Insurance coverage for harm from trial participation will be provided according to Partners healthcare policies

8. N/A we did not plan yet.

9. Suppl material: 17-34

BMJ Open

Protocol of a Randomized Controlled Trial in Cardiac Surgical Patients with Endothelial Dysfunction Aimed to Prevent Postoperative Acute Kidney Injury by Administering Nitric Oxide Gas

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Date Submitted by the Author:	02-May-2019
Complete List of Authors:	Marrazzo, Francesco; Massachusetts General Hospital , Department of Anesthesia, Critical Care and Pain Medicine Spina, Stefano; Massachusetts General Hospital, Department of Anesthesia, Critical Care and Pain Medicine Zadek, Francesco; Massachusetts General Hospital, Department of Anesthesia, Critical Care and Pain Medicine Sherpa Lama, Tenzing; Massachusetts General Hospital, Department of Anesthesia, Critical Care and Pain Medicine Xu, Changhan ; Massachusetts General Hospital, Department of Anesthesia, Critical Care and Pain Medicine Larson, Grant; Massachusetts General Hospital, Department of Anesthesia, Critical Care and Pain Medicine Rezoagli, Emanuele; Massachusetts General Hospital, Department of Anesthesia, Critical Care and Pain Medicine Malhotra, Rajeev; Massachusetts General Hospital, Department of Medicine, Cardiology Division Zheng, Hui; Massachusetts General Hospital, Department of Medicine Bittner, Edward; Massachusetts General Hospital, Department of Anesthesia, Critical Care and Pain Medicine Shelton, Kenneth; Massachusetts General Hospital, Department of Anesthesia, Critical Care and Pain Medicine Melnitchouk, Serguei; Massachusetts General Hospital, Department of Cardiac surgery Roy, Nathalie; Massachusetts General Hospital, Department of Cardiac surgery Sundt, Thoralf; Massachusetts General Hospital, Department of Cardiac surgery Riley, William; Massachusetts General Hospital, Department of Surgery, Cardiac Surgery, Perfusion Services Williams, Purris; Massachusetts General Hospital, Respiratory Care Services Fisher, Daniel; Boston Medical Center, Respiratory Care Services Kacmarek, Robert; Massachusetts General Hospital, Department of Respiratory Care; Harvard University, Department of Anesthesiology Thompson, Taylor; Massachusetts General Hospital, Department of Medicine, Pulmonary & Critical Care Unit Bonventre, Joseph ; Brigham and Women's Hospital Department of Medicine, Department of Medicine, Division of Renal Medicine

	Zapol, Warren; Massachusetts General Hospital, Department of Anesthesia, Critical Care and Pain Medicine Ichinose, Fumito; Massachusetts General Hospital, Department of Anesthesia, Critical Care and Pain Medicine Berra, Lorenzo; Massachusetts General Hospital, Department of Anesthesia, Critical Care and Pain Medicine
Primary Subject Heading:	Anaesthesia
Secondary Subject Heading:	Cardiovascular medicine, Renal medicine
Keywords:	Nitric Oxide, Hemolysis, Acute Kidney Injury, Cardiopulmonary Bypass, Endothelial Dysfunction

TITLE PAGE

Protocol of a Randomized Controlled Trial in Cardiac Surgical Patients with Endothelial Dysfunction Aimed to Prevent Postoperative Acute Kidney Injury by Administering Nitric Oxide Gas

Francesco Marrazzo[1], Stefano Spina[1], Francesco Zadek[1], Tenzing Sherpa Lama[1], Changhan Xu[1], Grant Larson[1], Emanuele Rezoagli[1], Rajeev Malhotra[2], Hui Zheng[3], Edward A. Bittner[1], Kenneth Shelton[1], Serguei Melnitchouk[4], Nathalie Roy[4], Thoralf M. Sundt[4], William D. Riley[5], Purris Williams[6], Daniel Fisher[7], Robert M. Kacmarek[6], Taylor B. Thompson[8], Joseph V. Bonventre[9], Warren M. Zapol[1], Fumito Ichinose[1], Lorenzo Berra[1]

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Protocol version: September 21, 2018

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Conflict of interest statement: Francesco Marrazzo and Lorenzo Berra salaries are partially supported by NIH/NHLBI 1 K23 HL128882-01A1. Joseph V. Bonventre is co-inventor on patents that are assigned to Partners Healthcare. Robert M Kacmarek is a consultant for Medtronic and Orange Medical and has received research grants from Medtronic and Venner Medical. None of the other authors has any conflict of interest to disclose.

Author Contributions: Authorship for this trial will be given to key personnel involved in trial design, personnel training, recruitment, data collection, statistical plan, and data analysis. There are no publication restrictions. LB, WMZ, JVB, TBT, NR, EAB, TMS, DF, ER, FM, and FI were responsible for conceptualizing trial design. LB managed patient safety protocol. FM, SS, FZ, CX, GL, and TSL are responsible for recruitment, enrollment, and data collection. RM, HZ, and EAB are responsible for power calculation, statistical plan and data analysis. LB, KS, SM, WDR, PW and RMK trained personnel for the clinical trial and built systems for Nitric Oxide delivery and monitoring. All authors have critically revised the study protocol and approved the final version. All authors agree to be accountable for the accuracy and integrity of all aspects of this trial.

Registration details: NCT02836899

Keywords: Nitric Oxide, Hemolysis, Acute Kidney Injury, Cardiopulmonary Bypass, Endothelial Dysfunction

Word count:

Abstract: 300

Text: 5830

Article Summary:

Strengths and limitations of this study:

- Supplementation with nitric oxide gas during and after surgery requiring prolonged cardiopulmonary bypass might be most beneficial to those patients with endothelial dysfunction who are unable to replace the hemolysis-mediated NO depletion due to an impaired endothelial nitric oxide synthase (eNOS) activity.

- The number of confounding variables resulting from the liberal use of nephrotoxic agents, the preoperative variability in serum creatinine level and the perioperative optimization of hemodynamics and volume status is minimized, since the “Kidney Disease: Improving Global Outcomes” (KDIGO) guidelines for renal protection are a standard of care at our institution.
- Results from this study could possibly be generalized to other clinical scenarios of intravascular hemolysis and vascular depletion of nitric oxide.
- This trial is designed to recruit patients from a single academic institution (Massachusetts General Hospital).

ABSTRACT

Introduction: Postoperative acute kidney injury (AKI) is a common complication in cardiac surgery. Levels of intravascular hemolysis are strongly associated with postoperative AKI and with prolonged (>90 minutes) use of cardiopulmonary bypass (CPB). Ferrous plasma hemoglobin released into the circulation acts as a scavenger of nitric oxide (NO) produced by endothelial cells. Consequently, the vascular bioavailability of NO is reduced, leading to vasoconstriction and impaired renal function. In patients with cardiovascular risk factors the endothelium is dysfunctional and cannot replenish the NO deficit. A previous clinical study in young cardiac surgical patients with rheumatic fever, without evidence of endothelial dysfunction, showed that supplementation of NO gas decreases AKI by converting ferrous plasma hemoglobin to ferric methemoglobin, thus preserving vascular NO. In this current trial we hypothesized that 24 hours administration of NO gas will reduce AKI following CPB in patients with endothelial dysfunction.

Methods and analysis: This is a single-center, randomized (1:1) controlled, parallel-arm superiority trial that includes patients with endothelial dysfunction, stable kidney function, and who are undergoing cardiac surgery procedures with an expected CPB duration >90 minutes. Patients are randomized to receive either nitrogen (N_2, control group) or 80 parts-per-million NO (intervention group). Test gases (N_2 or NO) are delivered during CPB and for 24 hours after surgery. The primary study outcome is the occurrence of AKI among study groups. Key secondary outcomes include AKI severity, occurrence of renal replacement therapy, major adverse kidney events at 6 weeks after surgery, and mortality. We are recruiting 250 patients, allowing detection of a 35% AKI relative risk reduction, assuming a two-sided error of 0.05.

Ethics and dissemination: The Partners Human Research Committee approved this trial. Recruitment began in February 2017. Dissemination plans include presentations at scientific

conferences, scientific publications, and advertising flyers and posters at Massachusetts General Hospital.

TEXT

Introduction

Acute kidney injury (AKI) is a common and serious complication after cardiac surgery procedures requiring prolonged (>90 minutes) cardiopulmonary bypass (CPB)[1–3]. Because post-cardiac surgical AKI is associated with increased risk of chronic kidney disease (CKD) and mortality[4–6], multiple interventions have been tested in large multicenter trials, but none have been successful at reducing AKI[7–10].

There are two major categories of risk factors for post-surgical AKI. The first is the patient's cardiovascular comorbidities that are associated with impaired endothelial function. These include obesity, diabetes, atherosclerosis, peripheral vascular disease, hyperlipidemia, smoking, and hypertension[2,11]. The second is the extent of CPB-associated hemolysis[12–15]. During hemolysis, plasma hemoglobin (Hb) is released into the circulation, and depletes plasma nitric oxide (NO)[16,17]. NO is a potent mediator of vasodilation and regulates blood flow to tissues[18]. In patients with endothelial dysfunction, the activity of endothelial NO synthase (eNOS), the enzyme responsible for NO production, is impaired and is unable to replenish the NO consumed by plasma Hb[19–21].

In an animal model of free water-induced intravascular hemolysis, Minneci et al. showed that hemolysis resulted in a reduction of creatinine clearance. The negative effects of hemolysis on renal function were attenuated by the inhalation of 80 parts-per-million (ppm) NO gas. NO oxidized plasma Oxy-Hb (Fe^{2+}) to ferric methemoglobin (Met-Hb, Fe^{3+}), thereby decreasing plasma NO consumption[16]**.** In a metanalysis, the administration of NO gas has been associated with increased risk of AKI in critically-ill patients with ARDS, especially with prolonged use[22]. However, no studies have shown an association between NO administration and increased incidence of AKI in pediatric or adult cardiac surgery population.

In a recent randomized controlled trial, administration of 80ppm NO gas for 24 hours, starting at the onset of CPB, was found to decrease postoperative AKI in a population of young Chinese cardiac surgical patients (average age 48), with no risk factors for endothelial dysfunction, who were undergoing cardiac valve replacement due to rheumatic heart disease[17]. NO administration also improved long-term outcomes such as Major Adverse Kidney Events (MAKE) and reduced stage 3 CKD for up to 1-year after surgery. However, it is still undetermined whether NO gas exerts similar, or even additional, renal protective effects in patients with endothelial dysfunction commonly treated in the United States.

Methods and analysis

Trial design

In this, single-center, randomized (1:1) controlled, parallel-arm superiority trial (RCT), we hypothesized that 24-hour administration of NO gas starting from the initiation of CPB will decrease AKI by converting Oxy-Hb to Met-Hb in patients with signs and symptoms of endothelial dysfunction suggestive of impaired activity of eNOS (primary outcome).

After screening, consented patients who meet the enrollment criteria are randomized to receive either 80ppm NO (intervention group, n=125) or Nitrogen (N_2, control group, n=125) for 24 hours as nitrogen is the major component of air and can be used as placebo. The test gas is administered via the oxygenator during CPB, then by inhalation once mechanical ventilation is resumed. When the patient is extubated, the test gas is delivered by high flow nasal cannula. On the day of surgery, after the insertion of a pulmonary artery catheter (PAC), patients are stratified by preoperative mean pulmonary artery pressure (i.e. mPAP <30 mmHg or mPAP ≥30 mmHg) then allocated into the two study groups. Consented patients with mPAP found to be

≥40 mmHg on the day of surgery after PAC placement, are not randomized in this study because NO or other pulmonary vasodilators might be indicated as part of the medical treatment during the perioperative period. Study outcomes are assessed during the hospital stay, at a 6-week follow-up visit, and by phone at 90 days and 1 year after the surgical procedure.

In order to avoid postoperative AKI, at our institution the "Kidney Disease: Improving Global Outcomes (KDIGO) guidelines" are the standard of care in all cardiac surgical patients[23]. The implementation of these guidelines has been shown to limit the incidence and severity of CPB-associated AKI[24]. Per these guidelines, patients do not receive any nephrotoxic agents such as intravenous contrast agents[25] during the first 48 hours after surgery. Patients receive close monitoring of serum creatinine and hourly urine output, and avoid intraoperative and postoperative hyperglycemia for at least 72 hours after surgery. Hemodynamics are closely monitored to maintain adequate cardiac output (CO) and blood pressure by using intraoperative transesophageal echocardiography (TEE) in combination with the PAC and a radial artery catheter[23,24]. The pulmonary and radial artery catheters are removed only after the patient is discharged from the intensive care unit (ICU) and after completion of test gas treatment.

The study enrollment opened in February 2017 and the first patient entered the trial in June 2017. The study team plans to enroll 250 patients over a period of 3 years. The study design is depicted in Figure 1.

Eligibility criteria

The study population includes men >40 years old and women >50 years old who have signs and symptoms of endothelial dysfunction, have stable kidney function in the 3 months prior to surgery, are undergoing cardiac surgery procedures with expected prolonged CPB (>90

minutes), and are not currently enrolled in other research studies. The presence of endothelial dysfunction is evaluated by using a brief questionnaire that integrates medical history with clinical and laboratory data that has previously been associated with endothelial dysfunction (Figure 2: Screening questionnaire to detect endothelial dysfunction)[26–35]. Eligible patients are those undergoing a scheduled procedure or patients who are already admitted to the hospital. Key exclusion criteria include estimated glomerular filtration rate (eGFR) <30 ml/min/1.73m^2, mPAP ≥40 mmHg and intravenous (I.V.) contrast infusion within 48 hours before surgery. A complete list of enrollment criteria is provided in Table 1.

Intervention

Perioperative management

Perioperative management including the implementation of the KDIGO guidelines has been shown to decrease the incidence of post-cardiac surgical AKI[24]. These guidelines recommend the avoidance of nephrotoxic agents, the close monitoring of serum creatinine and urine output, the avoidance of hyperglycemia, the consideration of alternatives to radiocontrast agents, and a close hemodynamic monitoring to maintain adequate cardiac output, blood pressure and peripheral oxygen delivery. To reduce AKI incidence related to associated factors during prolonged CPB, the KDIGO guidelines have been implemented at our institution as a standard of care in all patients undergoing cardiac surgery.

Anesthesia management of patients enrolled in the study is performed according to the hospital standard of care and using standardized protocols[36]. Before entering the operating room (OR), patients are pre-medicated with 2-5 mg of midazolam. Prophylactic antibiotics (cefazolin 2-3 g) are administered through a peripheral venous catheter (Introcan Safety® IV Catheter, B Braun). These are given during the hour before the surgical incision. In the OR, electrocardiography (EKG) and peripheral oxygen saturation (SpO_2) are continuously recorded. A radial arterial catheter (Arrow® Seldinger Arterial Catheter) is placed for continuous blood pressure

monitoring and blood sampling. Patients are then pre-oxygenated at an inspired oxygen concentration of 100% for 3 minutes. Anesthesia is then induced with an I.V. bolus injection of 4-6 µg/kg of fentanyl and either propofol 2 mg/kg or etomidate 0.1 mg/kg. Rocuronium 1.2 mg/kg or cisatracurium 0.2 mg/kg are used for paralysis prior to oro-tracheal intubation. Medication doses are adjusted in elderly patients. After intubation, patients are connected to the anesthesia machine, and mechanical ventilation is delivered in volume-controlled mode with a tidal volume of 6 ml/kg. Respiratory rate is titrated to maintain a partial pressure of carbon dioxide ($PaCO_2$) between 35-45 mmHg. The inspired fraction of oxygen (FiO_2) is titrated according to the partial pressure of oxygen (PaO_2), which is measured with arterial blood gas (ABG) analysis. The TEE probe (X7-2t 3D ultrasound probe, Philips) is inserted by the cardiac-anesthesia team for real-time intraoperative feedback to cardiac surgeons on (I) right and left ventricular function, (II) the mechanical stability and the function of repaired/replaced heart valves, and (III) the detection of air emboli/clots. The neck, chest, and abdomen are prepped and draped in a sterile fashion. A urinary catheter is positioned for hourly urinary output monitoring. To determine intraoperative and postoperative effects of NO on hemodynamics, after induction of anesthesia, a PAC (Edwards Lifescience, Irvine, CA) is placed in all patients for continuous hemodynamic monitoring of central venous pressure (CVP), pulmonary artery pressure (PAP), and cardiac index (CI). Electrodes for SEDLine brain function monitoring (Masimo®, Irvine, CA) are positioned on the forehead of each patient to assess the depth of anesthesia by displaying frontal cortex electroencephalogram waveforms and measuring the patient state index (PSI)[37]. The level of anesthesia is maintained by isoflurane administration (0.8-1.2 minimum alveolar concentration) and adjusted based on hemodynamics, while sustaining a PSI score between 30 and 50. Paralysis is maintained throughout the entire procedure by continuous infusion of rocuronium 4-10 µg/kg/min or cisatracurium 1-3 µg/kg/min. When clinically indicated, inotropes or vasopressors are administrated to maintain a mean arterial pressure (MAP) >65 mmHg and a CI >2.2 l/min/m^2.

The perfusion equipment utilized at Massachusetts General Hospital consists of Stockert S5 (LivaNova, Mirandola, Italy) heart-lung machines, CardioQuip (CardioQuip, Bryan, TX, USA) cooler-heaters, the LivaNova Xtra autologous cell salvage system and Medtronic HMS Plus Hepcon system (Medtronic, Minneapolis, MN, USA).

The CPB circuit is primed with 1600 ml Ringer's Lactate and recirculated for priming and air removal. Prior to cannulation, the patient is fully heparinized to a target ACT of no less than 400 seconds and a target heparin concentration of no less than 2.0 I.U./ml. The loading dose of heparin is calculated by the Hepcon device and subsequent ACT and heparin concentration assays are performed on the same system. The cannulation strategy may be central, peripheral or a combination of both depending on the type of surgery (coronary/valve vs. aortic), level of urgency, surgical approach (median sternotomy vs. thoracotomy), patient specific variables such as body habitus, previous cardiac surgical history, vascular disease and vascular anatomic anomalies. Most commonly, an arterial outflow cannula is placed in the distal ascending aorta and venous drainage is achieved by placing a multi-stage venous cannula in the right atrium via the inferior vena cava (IVC). The aortic root is typically cannulated with a catheter to deliver antegrade cardioplegia as well as to decompress the left ventricle. Retrograde catheters are commonly placed in the coronary sinus to maximize myocardial protection during the aortic cross clamp.

Once fully cannulated and prior to CPB, 500ml to 1000ml of prime volume may be removed from the CPB circuit via retrograde autologous priming (RAP) and/or venous antegrade priming (VAP) to reduce the crystalloid burden on the patient upon initiation of CPB. Once the patient is placed on CPB hypothermia is initiated to an extent dictated by type of surgery and surgeon's preference. Mild hypothermia (temperature maintained between 32-35°C) is most common for coronary and valve operations whereas circulatory arrest cases call for deeper hypothermia (temperature maintained between 18-24°C).

Aortic occlusion is achieved by application of an aortic cross clamp and myocardial protection is achieved by administration of either Del Nido cardioplegia[38] or a traditional 4:1 (blood:crystalloid) mixture at hypothermia into the aortic root (antegrade) and the coronary sinus (retrograde). Diastolic arrest is maintained by intermittent doses of cardioplegia every 20-30 minutes.

Blood flow rates while on CPB are maintained at a cardiac index (CI) of 2.4 l/min/m^2 or greater, a MAP of 65-75 mmHg and a urinary output of greater than 0.5 ml/kg/hr. To achieve these MAP goals phenylephrine may be titrated to effect and diuretics such as furosemide (10 mg bolus) may be given. Sweep gas flow and FiO_2 are regulated to maintain a PaO_2 of 150-250 mmHg, $PaCO_2$ of 35-45 mmHg and a mixed venous oxygen saturation (SvO_2) greater than 65%. Arterial and venous blood gases are drawn every 30 minutes and analyzed by the hospital laboratory. Ultrafiltration with a LivaNova hemoconcentrator (LivaNova, Mirandola, Italy) may be performed with or without maintaining zero balance fluid administration for the purposes of normalizing potassium levels (target K^+ 3.5-5.5 mmol/l) and to increase hematocrit (target hematocrit (HCT) greater than 21%), respectively. In the event ultrafiltration is inadequate to increase HCT to greater than 21% packed red blood cells or red blood cells processed by intraoperative autotransfusion may be administered via the LivaNova Xtra autologous cell salvage system. Target blood glucose levels of 100-180 mg/dL are maintained by administration of IV insulin (bolus 10 I.U. and continuous infusion of 2 I.U./hour) when the glucose level exceeds 180 mg/dL.

Once the procedure is complete, the heart and aorta are de-aired and the aortic cross clamp is removed. Temporary epicardial pacing leads are placed, the surgical site is policed for bleeding and the patient is weaned from CPB. Upon satisfactory termination from CPB, the cannulas are removed and the patient is administered protamine sulfate to reverse heparin anticoagulation at a dose indicated by the Hepcon system. Full heparin reversal is confirmed by a heparin level

assay and ACT measurement. Chest tubes are inserted and the sternum is approximated according to procedure and surgeon preference.

After surgery, patients are transferred to the cardiac surgical intensive care unit (CSICU). Standardized protocols for temperature, sedation, pain, glucose, and hemodynamic management are followed. Upon CSICU arrival, active rewarming using a forced-air warming device is performed. Propofol 2 mg/kg/h, ketamine 0.5 mg/kg/h, or dexmedetomidine 0.5-1.5 µg/kg/min are administered and titrated to achieve appropriate sedation. Pain control is achieved with the use of multimodal regimens (I.V. opioids, acetaminophen, and NSAIDS). Within the first 72 hours, blood glucose levels are monitored every 4 hours and I.V. insulin may be used to maintain glucose levels between 100 and 180 mg/dl. Volume status and hemodynamic parameters are continuously monitored throughout the patient CSICU stay by arterial line and PAC. If needed, vasopressors are administered to achieve the following hemodynamic targets: CI >2.2 l/min/m^2, MAP >65 mmHg, and SvO_2 >65%. Cardiac index, pulmonary vascular resistance (PVR), and systemic vascular resistance (SVR) are recorded every 4 hours until test gas suspension. Packed red blood cells (pRBCs) are transfused when Hb <7 g/dl[39]. Urinary output is recorded hourly via urinary catheter. The ICU team may administer furosemide I.V. in presence of oliguria only after optimization of hemodynamic parameters, volume status and assurance of no urinary catheter blockage. No nephrotoxic agents (e.g. I.V. contrast or hydroxyethyl starch products) are administered as recommended by the KDIGO guidelines[23].

Gas delivery

After PAC insertion, patients are randomly allocated to the intervention group (80ppm NO) or the control group (N_2). Using commercially available tanks of N_2 or NO (Airgas Inc, Radnor Township, Pennsylvania) and volumetrically-calibrated flowmeters, pure N_2 (placebo) or 850ppm NO gas in N_2 is mixed with pure O_2 or air. In the NO group, a final concentration of

80ppm NO is obtained. Test gas administration starts at the onset of CPB and lasts for 24 hours. The test gas is administered via the CPB machine into the sweep gas of the CPB oxygenator by regulating the tank as follows:

$$NO\ tank\ flow\ (L/min) = \frac{[sweep\ gas\ flow\ (L/min) \times NO\ concentration\ (80ppm)]}{NO\ tank\ concentration\ (850ppm)}$$

At the end of the CPB, once ventilation is resumed, test gas is delivered via the inspiratory limb of the anesthetic or mechanical ventilator circuit, and thereafter via the mechanical ventilator in the CSICU. The concentration of NO is regulated by using a Medical gas blender. When patients are extubated, they breathe test gas via a facemask or nasal prongs. NO and nitrogen dioxide (NO_2) levels are monitored through in-line NO/NO_2 sensors (Alphasense, Great Notley, Essex, UK). Met-Hb levels and oxygen saturation (SpO_2) are continuously measured using a peripheral pulse co-oximeter (Masimo Rainbow Set™ Technology, Irvine, CA). Schemas illustrating methods of NO and N_2 gas delivery and NO/NO_2 monitoring both during surgery and in the CSICU are reported in Figures 3 and 4.

Abrupt discontinuation of inhaled NO may lead to rebound pulmonary hypertension. This is characterized by worsening oxygenation and increased PAP, resulting in hypoxemia, systemic hypotension, bradycardia, and right ventricular failure[40–42]. For these reasons, after 24 hours, NO is weaned and discontinued while carefully monitoring hemodynamics using the PAC. NO weaning protocols at our institution consists of:

- Decreasing NO to a half dose and maintaining this dose for 5 minutes.
- Further decreasing NO dose to 5ppm and maintaining this dose for 5 minutes.
- Complete discontinuation of NO if no pulmonary hypertension rebound occurs and hemodynamics are stable for 10 minutes after NO gas suspension.

If at any time during the weaning protocol a patient experiences:

- Worsening hypotension (systolic blood pressure, SBP <90 mmHg) requiring the initiation of an I.V. infusion of vasopressor agents or, in patients already receiving vasopressors before NO weaning, a 50% increase of the dose of the vasopressors,
- Worsening hypoxemia requiring an FiO_2 increase of more than 0.2 or a positive end expiratory pressure (PEEP) increase of more than 5 cmH_2O;
- mPAP increase of more than 20% of the value measured before the initiation of the NO weaning, and/or
- A CI decrease below 1.5 L/min/m^2 in the presence of a mPAP over 25 mmHg,

a respiratory therapist (RT) resets the NO to the lowest level at which the patient was stable and notifies the principal investigator (P.I.) and the CSICU attending physician.

Failure of NO-weaning is established when one of the above complications occurs.

Blood and urine collection

To assess the extent of hemolysis and the renal-protective properties of NO administration, plasma samples for plasma free Hb, NO consumption, NO metabolites, and serum creatinine measurements are collected immediately before starting the surgical procedure, 15 minutes after the end of CPB, 24 hours after surgery, and 48 hours after surgery[15,17,43–45]. Additional blood samples for serum creatinine measurements are collected once a day for 7 days following the cardiac procedure or until discharge if the patient is discharged before day 7. Finally, a blood sample for serum creatinine is collected 6 weeks after surgery. Five urine samples are collected for urinary biomarkers of kidney injury (immediately before starting the surgical procedure, 15 minutes after the end of CPB, and at 24 hours, 48 hours and 6 weeks after surgery)[46–48]. After centrifugation, all specimens are coded, snap-frozen with liquid nitrogen, and stored on designated shelves in a -80°C freezer.

Outcomes

Primary outcome

The primary endpoint of this therapeutic trial is to determine whether there is a difference in AKI incidence between the control group (receiving N_2) versus the intervention group (receiving NO). AKI is defined by KDIGO criteria as an abrupt (within 48 hours) reduction in kidney function correlated to an absolute increase in serum creatinine of 0.3 mg/dL or more (≥26.5 µmol/L) or a percentage increase in serum creatinine of 50% or more (1.5-fold from baseline) at any time during the first 7 days after surgery or a urinary output <0.5 ml/Kg/h for >6 hours[49].

Secondary outcomes

Secondary renal outcomes include:

1. AKI severity using the KDIGO stages[49]:
    - Stage 1: Serum creatinine increase ≥26.5 µmol/l (≥0.3 mg/dl) or increase to 1.5-2.0-fold from baseline or urinary output <0.5 ml/kg/h for 6 hours;
    - Stage 2: Serum creatinine increase >2.0-3.0-fold from baseline or urinary output <0.5 ml/kg/h for 12 hours;
    - Stage 3: Serum creatinine increase >3.0-fold from baseline or serum creatinine ≥354 µmol/l (≥4.0 mg/dl) or urinary output <0.3 ml/kg/h for 24 hours or anuria for 12 hours or a need for renal replacement therapy (RRT).
2. Requirement for RRT following AKI during hospitalization and at 6 weeks, 90 days and 1 year after surgery.
3. Major Adverse Kidney Events (MAKE) at 6 weeks after surgery. MAKE is a composite outcome of death, new RRT, and worsened renal function (defined as a 25% or greater decline in eGFR)[50].

Secondary non-renal outcomes include:

1. Sequential organ Failure Assessment (SOFA) score during ICU stay[51].

2. Prolonged cardiovascular support defined as the need for vasopressors and inotropic agents, a balloon pump, or a ventricular-assist device for more than 48 hours after cardiac surgery.
3. Maximum hourly vasoactive-inotropic score (VIS) for the first 7 days after surgery and duration of vasopressors and inotropic agents support. VIS is calculated as dopamine dose (mcg/kg/min) + dobutamine dose (mcg/kg/min) + 100 x epinephrine dose (mcg/kg/min) + 10 x milrinone dose (mcg/kg/min) + 10,000 x vasopressin dose (units/kg/min) + 100 x norepinephrine dose (mcg/kg/min) + 10 x phenylephrine dose (mcg/kg/min)[52,53].
4. Duration of mechanical ventilation (hours).
5. ICU and hospital length of stay

Exploratory outcomes

1. Severity of AKI using urinary biomarkers of kidney injury[46–48].
2. AKI incidence and severity related to baseline characteristics of enrolled patients such as the presence of eGFR <60 ml/min at baseline, mPAP at baseline, cardiovascular risk factors associated with endothelial dysfunction, the type of surgical procedure, EuroSCORE II[54]) and intraoperative course (i.e. duration of CPB, duration of aortic cross-clamp, levels of plasma free Hb, levels of NO consumption and NO metabolites) that may impact study results.
3. Delirium assessed using the confusion assessment method for the intensive care unit (CAM-ICU) within the first 7 days after surgery or until ICU discharge[55,56].
4. Quality of life at 6 weeks, 90 days, and 1 year after surgery assessed using the Katz Index of Independence in Activities of Daily Living (ADL) and the PROMIS global health questionnaire[57–59].

Safety outcomes

1. Intra-hospital mortality and mortality at 6 weeks, 90 days, and 1 year after surgery.
2. Non-fatal stroke during hospitalization and at 6 weeks after surgery.
3. Perioperative and non-perioperative non-fatal myocardial infarction[60].
4. Postoperative bleeding calculated as the sum of blood loss through thoracic drains from the moment of chest closure over a period of 24 hours.
5. Need for blood transfusions or transfusions with blood products
6. Postoperative infections (e.g., pneumonia, wound infection, endocarditis, central line infection, urinary tract infection, sepsis).
7. Cardiac arrhythmias and other non-cardiac postoperative complications (e.g., hepatobiliary disorders, pneumothorax, pleural effusion, vascular disorders).
8. Patients requiring a decrease of NO delivery due to Met-Hb >5%.

Sample size planning

The sample size needed for this trial is calculated based on the primary endpoint: the reduction of AKI incidence in the NO treated group (intervention group) compared to the N_2 group (control group). In a randomized controlled trial (RCT) conducted at the University of Muenster (Germany), Meersch et al. showed that implementation of a KDIGO CT surgery "bundle" as recommended by the KDIGO guidelines successfully reduced the incidence of CPB-associated AKI from 71.7% to 55.1%[24]. At Massachusetts General Hospital (MGH), the use of the KDIGO guidelines is part of standard care and the CPB-associated AKI rate is similar (55% as observed in a one-year chart review performed by the investigators for surgery requiring CPB >90 minutes [data not shown]). In a prior trial we conducted in China, we found a 22% relative risk reduction in the incidence of AKI and a 42% relative risk reduction of stage 3 CKD at 1 year in the NO treated group[17]. In this study, we estimate a greater reduction in the incidence of AKI (35% relative risk reduction) because we anticipate that an American population with endothelial dysfunction will benefit more from NO treatment. Thus, in the NO group, the incidence of AKI is

expected to decrease from 55% to 35.75%. Using a Fisher's exact test to compare proportions, the sample size needed to detect a difference, assuming a two-sided type I error of 0.05 and a power of 0.8, is 114 patients per group. In order to account for possible dropouts, we have increased our sample size by 10%. The enrollment plan is 250 patients (125 patients in the NO group and 125 patients in the N_2 group).

Recruitment

This study is performed at MGH in Boston, MA. All inpatients and oupatients undergoing a scheduled procedure are screened for eligibility. If a patient is excluded, the reasons are noted on a screening log. To obtain consent from scheduled patients, the details of the study are given during the preoperative visit at the Cardiac Surgery Preoperative Clinic, along with a flyer overviewing the trial protocol and a copy of the consent form. For patients admitted to the hospital, study details are given at least a day prior to their cardiac procedures. On the day of surgery, prior to the initiation of any study procedures, the patient's written consent is obtained by a licensed physician who is also a member of the study staff. Consent is obtained exclusively from the patient, and not from a surrogate. Patients who choose not to participate in this study receive standard care according to the surgical procedure. After consent is obtained, a de-identified code is assigned to the patient and registered on a dedicated enrollment log.

Randomization and treatment allocation

To ensure balance between study groups with respect to the likelihood of receiving NO after surgery, after the insertion of a PAC, the patients are stratified by preoperative mPAP (i.e. mPAP <30 mmHg or mPAP ≥30 mmHg) then allocated into the two study groups. The randomization sequence is created by an independent statistician using Stata Statistical Software: Release 14 (College Station, StataCorp LP, TX, USA) and then uploaded on the MGH Research Electronic Data Capture (REDCap) application[61]. A predetermined block

randomization method (fixed block size of 10) is used to ensure equal distribution of participants to treatment arms. A physician with no clinical involvement in providing care for the enrolled patients will conduct the randomization procedure throughout the study by using the randomization function provided by REDCap. Thus, the randomization is conducted without any influence from clinicians providing care for the patients, or from statisticians or outcome assessors.

Blinding

The NO and N_2 tanks and the gas delivery systems in the OR and at the bedside are masked and cannot be distinguished on the basis of appearance. This allows to keep participants, clinicians, and investigators blind to the assignment group. For safety and gas monitoring, the clinician administering the test gas remains unblinded to the treatment. This unblinded clinician is solely responsible for gas tank preparation and test gas delivery and monitoring. Assessors who are blind to the treatment allocation conduct assessment of study outcomes. Blinding is maintained until 1 year follow up. The allocation of patients enrolled in the study will not be disclosed before completion of the study (250 patients enrolled). To maintain the overall quality, legitimacy, and integrity of the clinical trial, unblinding of the test gas may occur only in critical circumstances when the responding physician prescribes initiation of NO. In this circumstance, the P.I. fully documents and explains the reasons for unblinding in a report to the Institutional Review Board (IRB).

Data analysis

All trial outcomes will be evaluated using an intention-to-treat analysis plan. Hypothesis testing will be performed using a two-sided significance level (type I error) of α=0.05. Sensitivity analyses per group of treatment will also be performed and compared to the intention-to-treat analysis results. The incidence of AKI (primary endpoint) and postoperative adverse events

after prolonged CPB will be reported in terms of relative risk with 95% confidence interval (CI) in the treatment versus the control group. The median differences (NO group versus control group) with 95% CI will describe differences in continuous perioperative characteristics and continuous postoperative outcomes.

An unadjusted analysis of the primary endpoint will be performed by means of Fisher's exact test comparing incidence of AKI in the NO group against the control (placebo) group. Multivariate logistic regression will be performed to determine if treatment with NO reduces incidence of AKI after adjusting for eGFR, age, sex, EUROscore II, and CPB time.

Secondary and exploratory endpoints will be addressed using appropriate tests for each outcome. Categorical variables will be compared between the two groups using Fisher's exact test or Chi square as appropriate. Continuous variables will be compared between the two groups using student's t-test or a Mann-Whitney U test as appropriate. The change in the laboratory markers over time and between the two groups will be tested with a mixed effects modeling. Differences among the groups at each timepoint will be tested using an unpaired student's t-test or a Mann-Whitney U test as appropriate.

Interim analysis

An interim analysis by a Data Safety Monitoring Board (DSMB) is planned upon reaching 50% of the study population. The DSMB is comprised of three independent, multidisciplinary experts (online supplementary material: Data and Safety Monitoring Board section). An independent statistician will perform the interim analysis. The statistician will report the results of the data analyzed to the DSMB in a closed session. The DSMB will have access to data collected during the hospital stay and during the follow up visits, including maintenance of patient confidentiality throughout the study. The DSMB may recommend terminating the study if at the interim analysis a significant increase of intra-hospital mortality, mortality at 6 weeks, AKI or need for RRT in either group (N_2 or NO) is detected.

Data management

All data collected for the trial are entered into the MGH Research Electronic Data Capture (REDCap) application[61]. Preoperative, intraoperative, and postoperative data are prospectively collected from the patient's chart until discharge. Follow-up data are collected by interviewing patients at 6 weeks, 90 days and 1 year after surgery.

Data access is restricted and granted by the P.I. only to authorized members of the study team. The P.I. also assigns specific privileges to members of the study team for data import/export. Quality and integrity of the data collected are optimized by using software properties such as logic checks and validation of data fields (i.e. reference range, valid or invalid values). Warnings and error messages alert the co-investigator of missing data or data entries that do not match the requirements set up in the data-entry field. Also, the co-investigators manually perform weekly checks to ensure data consistency. During these periodical checks, all errors detected by the study team related to data collection and data entry are summarized in a data query report. The report is sent to the P.I. following the enrollment of every 5 patients. It is the responsibility of the P.I. to check the original forms for inconsistency, make corrections by modifying the original forms when necessary, and enter a response to the query. Strict confidentiality is maintained by the research team at all times. All forms are and will be kept in a secure, locked cabinet with limited access for at least 5 years after study completion.

Trial management

Study team physicians are responsible for screening all inpatients and outpatients undergoing a scheduled procedure. The number of eligible, consented, enrolled, and randomized patients is recorded in addition to the reasons for non-participation in the trial.

During their hospital stay, patients are closely monitored and all outcomes are recorded. For this reason, no missing endpoints are expected. After discharge, all patients have a scheduled

follow-up visit with the cardiac surgeon 6 weeks after surgery. The day before their appointment, the patient is contacted by a member of the study staff to remind them that a member of the team will collect research data during their visit. This is done by a phone call (or e-mail if unreachable by phone). At the end of the visit with the cardiac surgeon, blood and urine samples are collected for kidney function assessment and a questionnaire regarding medical and surgical complications and quality of life is given. Patients are then called at 90 days and 1 year after surgery to evaluate mid-term and long-term outcomes. To avoid missing follow-ups, a minimum of three calls are attempted by the study team. Calls are made at different times and dates in an attempt to increase the probability of contacting patients. Phone numbers provided by the patient on the screening day are used. If the study team is still unable to contact the patient despite the several phone call attempts, a letter is sent to the home address provided by the patient at their initial screening visit. If all attempts do not provide contact with the patient, a member of the study team may call to the patient's primary care physician or another healthcare provider to obtain information regarding the patient's condition (e.g. deceased). Based on trials previously performed in the Department of Surgery at the MGH, we expect a loss to follow-up of 10%, 15%, and 20% at 6 weeks, 90 days, and 1 year respectively.

Trial risks

Due to the instability of NO, there are risks associated with its use that must be considered. NO reacts slowly with oxygen to form NO_2, which may cause airway inflammation and damage to lung tissues[62]. Moreover, NO oxidizes ferrous Hb to form Met-Hb, which is unable to transport and release oxygen to tissues. However, cyanosis in healthy patients does not appear until Met-Hb levels are 15-20%[63]. The binding of NO to Hb is a rapidly reversible reaction, with a half-life of 15-20 minutes after NO discontinuation. The side effects and adverse events related to NO delivery are well reported in literature. In Table 2 (Summary of Adverse Events from Previous Studies Examining Nitric Oxide), we summarized 8 clinical trials that recorded and reported

adverse events associated with NO gas in newborn, pediatric, and adult populations. Based on the present literature and FDA reports[64], the risks of breathing NO at 80ppm for 24 hours are minimal when Met-Hb levels and NO/NO_2 delivery levels are carefully monitored[65,66]. To improve safety, in the present trial NO is administered and monitored by trained respiratory therapists. The IRB for this study requested monitoring and maintaining levels of NO_2 breathing below 5ppm. We previously tested and found that our delivered circuits do not exceed delivering 1.5ppm of NO_2 when 80ppm of NO gas is delivered at 90% of FiO_2. Met-Hb is continuously monitored by non-invasive co-oximetry. If Met-Hb levels exceed 5% of circulating Hb, the concentration of NO delivered is halved from 80ppm to 40ppm and closely monitored until a reduction occurs. If Met-Hb levels persist above 5%, NO is progressively halved until a reduction below 5% occurs.

Criteria for patient discontinuation

The criteria for patient discontinuation from the study are:

- Voluntary discontinuation by a patient;
- Exiting the protocol for safety reasons based on the judgment of the clinical or research staff. Specific safety reasons include: (1) acute worsening of hypotension defined as a decrease in MAP of >20 mmHg that is not attributable to other causes (such as hypovolemia, hemorrhage or sepsis), but is generally due to worsening of left ventricular failure, (2) sudden worsening of hypoxemia defined as a decrease of SpO_2 to <80% while breathing 100% oxygen and not attributable to other causes (such as pulmonary edema, ARDS, or pulmonary embolism), and (3) an increase in NO_2 levels >5ppm from baseline.

Patient and public involvement

Patients were not directly involved in the study design and recruitment, and did not contribute to the development of the research questions and outcomes. At the end of the trial, upon request, results will be provided to the study participants and/or their family members and then published on clinicaltrtials.gov.

Enrollment to date

As of September 2018, 88 patients have been enrolled in the study. Assuming that we recruit about 8 patients per month, we anticipate completing the recruitment of 250 patients by May 2020.

Ethics and dissemination

The current trial was approved by the International Review Board (IRB) in August 2016. Further amendments of the study protocol have been performed and clinical trials.gov has been updated accordingly. Analysis of the outcomes of the study (primary, secondary and exploratory outcomes) will be performed when the enrollment of 250 patients is complete. These results will be published in a primary paper, which will be directed to a peer-reviewed journal and may be presented as a poster and/or through oral communication at scientific conferences focused on cardiac surgery, nephrology, anesthesia, and critical care medicine.

Conclusion

This trial is evaluating whether 24-hour administration of NO gas reduces the incidence of AKI after prolonged CPB in patients with signs and symptoms of endothelial dysfunction. The results obtained by this trial may also represent further progress of the scientific community in evaluating the systemic beneficial properties of exposing plasma to NO gas.

Aknowledgements

The clinical perfusionists of the Department of Cardiac Surgery, Perfusion Services, Massachusetts General Hospital (Boston, MA, USA).

The respiratory therapy staff, the nursing staff and the physicians of the department of Anesthesia, Critical Care and Pain Medicine, Massachusetts General Hospital (Boston, MA, USA).

The nursing staff and the physicians of the department of Cardiac Surgery, Massachusetts General Hospital (Boston, MA, USA).

The nursing staff of the pre-admission testing area, Massachusetts General Hospital (Boston, MA, USA).

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TABLES

Inclusion criteria:

1. Age ≥ 18 years of age
2. Elective cardiac or aortic surgery requiring CPB and with expected CPB > 90 minutes
3. Clinical evidence of endothelial dysfunction assessed by a specifically designed questionnaire (Figure 2)
4. Stable preoperative renal function without evidence of a plasma creatinine increase of ≥ 0.3 mg/dL within 3 months of study entry and without receiving RRT

Exclusion Criteria:

1. eGFR less than 30 ml/min/1.73 m^2
2. Emergent cardiac surgery
3. Life expectancy < 1 year at the time of enrollment
4. mPAP ≥ 40 mmHg and PVR > 4 Wood Units
5. LVEF < 30% by echocardiography obtained within 3 months of enrollment
6. Hemodynamic instability on the day of surgery as defined by a SBP <90 mmHg
7. Administration of one or more pRBC transfusion in the week prior to enrollment
8. X-ray contrast infusion less than 48 hours before surgery
9. Evidence of intravascular or extravascular hemolysis from any other origin:
    - Intravascular: Intrinsic RBC defects leading to hemolytic anemia (eg, enzyme deficiencies, hemoglobinopathies, membrane defects). Extrinsic: liver disease, hypersplenism, infections (eg, bartonella, babesia, malaria), treatment with oxidizing exogenous agents (eg, dapsone, nitrites, aniline dyes), exposure to other hemolytic agents (eg, lead, snake and spider bites), lymphocyte leukemia, autoimmune hemolytic disorders
    - Extravascular: Infection (e.g., clostridial sepsis, severe malaria), paroxysmal cold hemoglobinuria, cold agglutinin disease, paroxysmal nocturnal hemoglobinuria, iv infusion of Rho (D) immune globulin, iv infusion of hypotonic solutions

Table 1. Inclusion and exclusion criteria. CPB: Cardio-pulmonary Bypass; RRT: Renal replacement therapy; eGFR: estimated Glomerular Filtration Rate (CKD-EPI formula)[67]; mPAP: Mean pulmonary artery pressure; PVR: Pulmonary vascular resistance; LVEF: Left ventricular ejection fraction; SBP: Systolic blood pressure; pRBC: packed red blood cells

Authors	Number of Patients	Age Group (newborn, pediatric, adult)	NO (ppm)	Time of Exposure	Adverse Events
Van Meurs et al.[68]	210	Premature newborns <34 weeks gestational age (26 ± 2 gestational weeks)	5-10	10-14 hours	NO_2 >5ppm in 2 infants; Met-Hb >8% in 1 infant. No resulting complications were reported
Stork et al.[69]	114	Newborn >34 gestational weeks and <14 days old (39.3 ± 1.8 gestational weeks)	20-80	<14 days	Met-Hb >5% in 11 infants. No resulting complications were reported. A dose of 100ppm NO was given to two infants for 36 and 60 minutes, respectively. NO_2 levels in the second infant elevated to 5.1ppm. NO_2 and Met-Hb levels decreased after NO concentration returned to 80ppm
Roberts et al.[70]	30	Newborns >37 gestational weeks (39.8 ± 1.5 gestational weeks)	80	<8.5 days	Met-Hb elevated to 18.2% in 1 infant. The later clinical course was uneventful
James et al.[71]	101	Pediatric (1-43 months old)	20	1-3 hours	None related to NO delivery
Cueto et al.[72]	40	Pediatric (15 days old - 17 years old)	4-30	hours to days	Rebound effects of NO withdrawal reported in 2 patients
Lei et al.[17]	117	Adult >18 years old (48.7 ± 9.5 years old)	80	24 hours	None related to NO delivery
Rossaint et al.[73]	9	Adult (17-46 years old)	5-20	3-53 days	None related to NO delivery
Taylor et al.[74]	192	Adult >18 years old (50 ± 17 years old)	5	<28 days	66 reported infections in the NO group and 41 in the control group. Infections deemed unrelated to NO gas administration. No further complications were reported

Table 2. Summary of prior selected studies that reported adverse events related to nitric oxide administration. Data of age are presented as mean ± SD or range.

FIGURES

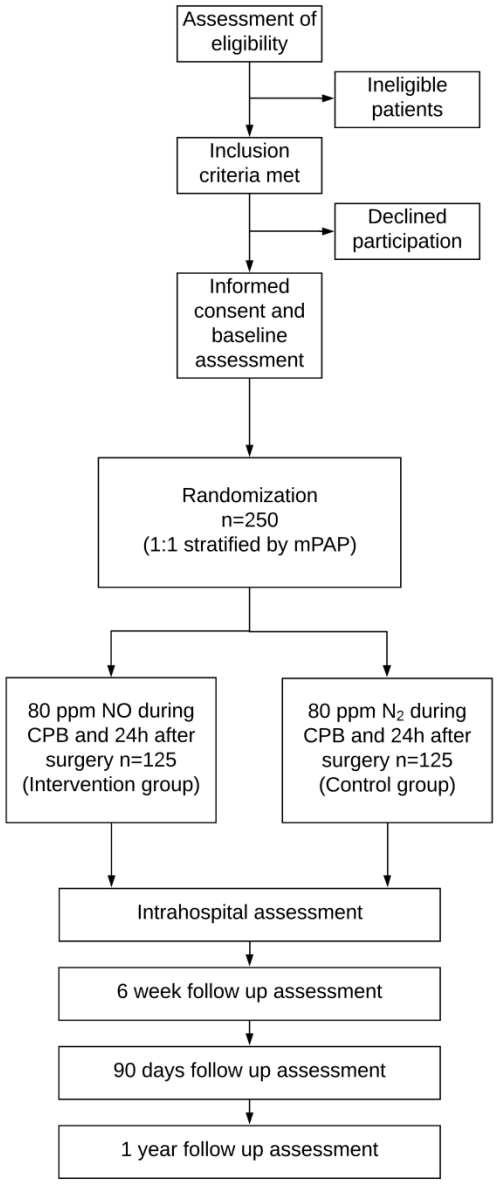
Figure 1. **Study design.** NO: Nitric Oxide; CPB: cardio-pulmonary bypass; N_2: Nitrogen; mPAP: mean pulmonary artery pressure. After placement of pulmonary artery catheter, to ensure balance between study groups with respect to the likelihood of receiving NO after surgery, patients are randomized based on mPAP measured by the pulmonary artery catheter placed on the day of surgery (mPAP <30mmHg and mPAP between 30 mmHg and 39 mmHg).

Figure 2. Screening questionnaire to detect endothelial dysfunction. The questionnaire above aims to systematically detect endothelial dysfunction in patients undergoing a cardiac surgical procedure. If "yes" is answered to at least 1 of the above questions, the patient can be considered to have endothelial dysfunction and he/she may be enrolled in the study. PTCA: Percutaneous transluminal coronary angioplasty; IDDM: Insulin dependent diabetes mellitus; NIDDM: Non-insulin dependent diabetes mellitus; LDL: Low-density lipoprotein; BMI: Body mass index; SBP: Systolic blood pressure;

Figure 3: Schema of the NO delivery systems in the operating room. A. Figure illustrating how the NO or N_2 is delivered into the CPB oxygenator. Tanks of pure N_2 are used in the control group and tanks of 850ppm NO in N_2 are used in the intervention group. A "Y" adaptor is inserted into the sweep gas input line leading to the oxygenator. This allows for the mixing of the test gas with the sweep gas (O2 + medical air). This mixture is periodically monitored with an NO/NO_2 analyzer directly before entering the oxygenator. **B.** Figure illustrating how the NO or N_2 is delivered into the anesthesia ventilator once ventilation has been resumed. The test gas is delivered by placing a "Y" adaptor into the inspiratory limb of the circuit. The mixture is scrubbed of NO_2 by a large volume scavenger containing calcium hydroxide and is periodically analyzed with a NO/NO_2 analyzer before being inhaled by the patient.

Figure 4: Schema of the NO delivery systems in the cardiac surgical intensive care unit.

A. Figure illustrating how the NO or N_2 is delivered through the mechanical ventilator at bedside in the ICU. Tanks of pure N_2 are used in the control group and tanks of 850ppm NO in N_2 are used in the intervention group. Test gas is blended with medical air and enters the air inlet of the ventilator. The high pressure O_2 hose is directly connected to the ventilator. If there is any change of FiO_2, the amount of NO/N_2 delivered is regulated by the RT by adjusting the blender setting and the ventilator FiO_2 setting insuring that the patient is still receiving the target concentration of 80ppm NO. The mixture obtained is then scrubbed of NO_2 through a large volume scavenger and a small volume scavenger placed in series on the inspiratory limb of the circuit. The final amount of NO and NO_2 delivered is periodically analyzed with a NO/NO_2 analyzer directly before the mixture is inhaled by the patient. **B.** Figure illustrating how the NO or N_2 is delivered into the High Flow Nasal Cannula device. The test gas is delivered to the system by placing "Y" adaptor before the humidifier. A commercially available blender mixes O2 and medical air and is regulated by the RT to reach the target FiO_2. The flow of NO_2 or N_2 is titrated to reach the desired concentration 80ppm NO or placebo. This mixture is then humidified and heated to a temperature of 34°C.



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131x295mm (600 x 600 DPI)

CABG and/or valve repair/replacement and age > 40 year old for males and > 50 year old for females and 1 out of 8 of the following criteria:	
Previous coronary artery bypass graft or PTCA (+ stent)	Yes___ No___
History or presence of intermittent claudication, critical limb ischemia, or peripheral vascular disease with the exception of vasculitis.	Yes___ No___
History of transient ischemic attack and/or ischemic stroke	Yes___ No___
Diagnosis of diabetes (IDDM or NIDDM) requiring oral hypoglycemic agents or insulin	Yes___ No___
Hypercholesterolemia (total cholesterol > 200 mg/dl or LDL > 160 mg/dl) treated with statins, ion-exchange resins or other oral agents	Yes___ No___
BMI > 40	Yes___ No___
Hypertension (SBP 140 ≥ mmHg) treated with antihypertensive drugs	Yes___ No___
Active smoking ≥ 10 pack - years	Yes___ No___

Figure 2. Screening questionnaire to detect endothelial dysfunction. The questionnaire above aims to systematically detect endothelial dysfunction in patients undergoing a cardiac surgical procedure. If "yes" is answered to at least 1 of the above questions, the patient can be considered to have endothelial dysfunction and he/she may be enrolled in the study. PTCA: Percutaneous transluminal coronary angioplasty; IDDM: Insulin dependent diabetes mellitus; NIDDM: Non-insulin dependent diabetes mellitus; LDL: Low-density lipoprotein; BMI: Body mass index; SBP: Systolic blood pressure;

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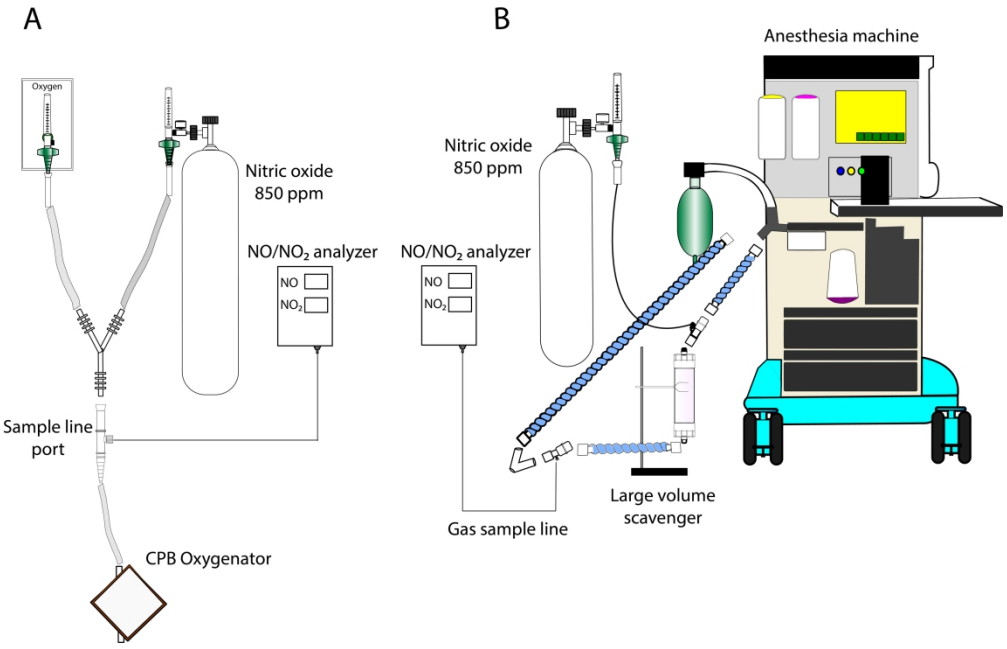

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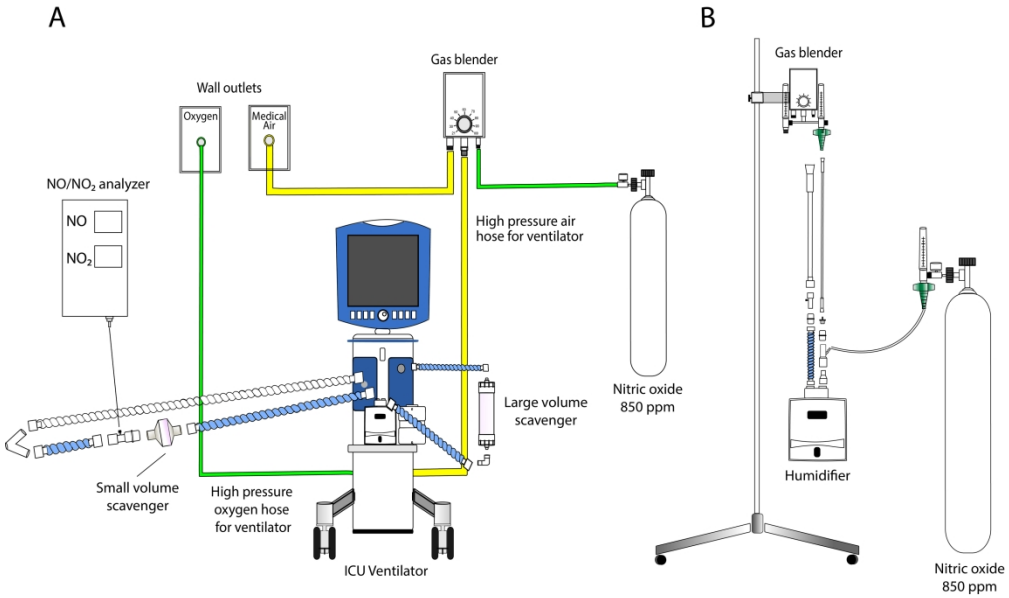

Figure 4: Schema of the NO delivery systems in the cardiac surgical intensive care unit. A. Figure illustrating how the NO or N2 is delivered through the mechanical ventilator at bedside in the ICU. Tanks of pure N2 are used in the control group and tanks of 850ppm NO in N2 are used in the intervention group. Test gas is blended with medical air and enters the air inlet of the ventilator. The high pressure O2 hose is directly connected to the ventilator. If there is any change of FiO2, the amount of NO/N2 delivered is regulated by the RT by adjusting the blender setting and the ventilator FiO2 setting insuring that the patient is still receiving the target concentration of 80ppm NO. The mixture obtained is then scrubbed of NO2 through a large volume scavenger and a small volume scavenger placed in series on the inspiratory limb of the circuit. The final amount of NO and NO2 delivered is periodically analyzed with a NO/NO2 analyzer directly before the mixture is inhaled by the patient. B. Figure illustrating how the NO or N2 is delivered into the High Flow Nasal Cannula device. The test gas is delivered to the system by placing "Y" adaptor before the humidifier. A commercially available blender mixes O2 and medical air and is regulated by the RT to reach the target FiO2. The flow of NO2 or N2 is titrated to reach the desired concentration 80ppm NO or placebo. This mixture is then humidified and heated to a temperature of 34°C.

285x169mm (300 x 300 DPI)

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Primary Registry and Trial Identifying Number	**ClinicalTrials: NCT02836899**
Date of Registration	July 19 2016
Secondary Identifying Numbers	IRB ID#: 2016 P001629
Source(s) of Monetary Support	National Heart, Lung, and Blood Institute (NHLBI) (Award Reference Number K23 HL128882-01A1)
Primary Sponsor	Department of Anesthesiology, Critical care and Pain Medicine Massachusetts General Hospital Boston, MA 02114
Contact name	Lorenzo Berra, MD lberra@mgh.harvard.edu 617-724-2000 Department of Anesthesiology, Critical care and Pain Medicine Massachusetts General Hospital

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Public Title	Effect of Nitric Oxide in Cardiac Surgery Patients With Endothelial Dysfunction
Scientific Title	Prevention of Acute Kidney Injury by Nitric Oxide in Prolonged Cardiopulmonary Bypass: A Double-Blind Controlled Randomized Trial in Cardiac Surgical Subjects with Endothelial Dysfunction
Countries of Recruitment	United States
Health Condition(s) or Problem(s) Studied	Cardiopulmonary bypass associated-Acute Kidney Injury

Intervention(s)	Trial arm 1: Nitric oxide (NO) Group (intervention arm). NO gas 80 parts-per-million is administered via the oxygenator during CPB, then by inhalation when mechanical ventilation is resumed. Once the subject is extubated, NO gas is delivered by high flow nasal cannula.The treatment begins at the onset of the cardiopulmonary bypass and lasts 24h. Trial arm 2: Nitrogen (N_2) Group (Control arm). N_2 gas is administered via the oxygenator during CPB, then by inhalation when mechanical ventilation is resumed. Once the subject is extubated, N_2 gas is delivered by high flow nasal cannula. The N_2 administration begins at the onset of the cardiopulmonary bypass and lasts 24h.
Trial Type	Interventional Allocation: Randomized Intervention model: Parallel assignment Blinding: Clinicians blinded to intervention, participants blinded to intervention, study

	investigators blinded to intervention, primary outcome assessor blinded to intervention. For safety and gas monitoring, only the clinician administering the test gas remains unblind to the treatment. This clinician is not part of the anesthesia, ICU, or surgical physician team delivering care. Assignment: Parallel Primary purpose: Prevention
Date of First Enrollment	June, 2017
Target Sample Size	250 patients
Recruitment Status	Recruiting
Key Enrollment Criteria	Inclusion criteria: Age ≥ 18 years of age; Elective cardiac or aortic surgery with expected CPB > 90 minutes; Clinical evidence of endothelial dysfunction assessed by a specifically designed questionnaire; Stable preoperative renal function without evidence of a plasma creatinine increase of ≥ 0.3 mg/dL within 3 months of study entry and without receiving RRT. Key exclusion criteria:

	estimated glomerular filtration rate (eGFR) <30 ml/min/1.73m^2, mPAP ≥40 mmHg and intravenous (I.V.) contrast infusion within 48 hours before surgery.
Primary Outcome(s)	Outcome name: Incidence of Acute Kidney Injury (AKI) Method of measurement: KDIGO criteria Time points of interest: 7 days after surgery
Key Secondary Outcomes	Outcome name: AKI severity Method of measurement: KDIGO stages Time points of interest: 7 days after cardiac surgery Outcome name: Renal Replacement Therapy Method of measurement: Medical record review Time points of interest: Up to 1 year. Outcome name: Major Adverse Kidney Events (MAKE) Method of measurement: Medical record

review

Time points of interest: 6 weeks after cardiac surgery.

Outcome name: Organ dysfunction (SOFA score)

Method of measurement: Medical record review

Time points of interest: ICU stay (up to seven days)

Outcome name: Prolonged cardiovascular support

Method of measurement: Medical record review

Time points of interest: 48 hours after cardiac surgery

Outcome name: duration of mechanical ventilation

Method of measurement: Medical record review

Time points of interest: up to 6 weeks

Outcome name: Intensive care unit length of stay (ICU-LOS)

Method of measurement: Medical record review

Time points of interest: up to 6 weeks

Outcome name: Hospital length of stay (LOS)

Method of measurement: Medical record review

Time points of interest: up to 1 year

Data and Safety Monitoring Board:

DSMB Chair:

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Responsibilities include: reviewing and evaluating the trial data to ensure participant safety, trial conduct, progress, and efficacy, and making recommendations regarding the continuation, or termination of the trial.

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

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

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

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

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

Chan A-W, Tetzlaff JM, 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
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	3
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	See note 1
Protocol version	#3	Date and version identifier	2
Funding	#4	Sources and types of financial, material, and other support	2
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	See note 2
Roles and responsibilities:	#5b	Name and contact information for the trial sponsor	See note 3

sponsor contact information			
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	See note 4
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)	See note 5
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	7,8
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	8
Objectives	#7	Specific objectives or hypotheses	8,9
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)	8
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, 19, 20
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)	9, 10, 35, 36
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10-16

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)	25
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	23-24
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	23
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	16-19
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, 24
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	19, 20
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	19, 20
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	20, 21
Allocation concealment	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed	20,21

mechanism		envelopes), describing any steps to conceal the sequence until interventions are assigned	
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	20, 21
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	21
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	21
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	22-24
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	22-24
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	22-24
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	21,22
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	21,22
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)	21,22

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	22 and suppl 16
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	22
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	21, 24, 25
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	22, 23
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	21, 26
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)	26
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	20
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	See note 6
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	22-24
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	2

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	22, 23
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	See note 7
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	26
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	See note 8
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	See note 9
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	16

Author notes

1. Suppl material: 9-15
2. Page 1, 3 and suppl material page 1-9
3. Suppl material: 9
4. N/A No role of the study sponsors and funders.
5. Suppl material: 16
6. To participate in this trial, one consent is collected. As indicated in question #33: We plan for collection, laboratory evaluation, and storage of biological specimens analysis in the current trial for possible ancillary studies.

7. Insurance coverage for harm from trial participation will be provided according to Partners healthcare policies

8. N/A we did not plan yet.

9. Suppl material: 17-34

BMJ Open

Protocol of a Randomized Controlled Trial in Cardiac Surgical Patients with Endothelial Dysfunction Aimed to Prevent Postoperative Acute Kidney Injury by Administering Nitric Oxide Gas

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Primary Subject Heading:	Anaesthesia
Secondary Subject Heading:	Cardiovascular medicine, Renal medicine
Keywords:	Nitric Oxide, Hemolysis, Acute Kidney Injury, Cardiopulmonary Bypass, Endothelial Dysfunction

TITLE PAGE

Protocol of a Randomized Controlled Trial in Cardiac Surgical Patients with Endothelial Dysfunction Aimed to Prevent Postoperative Acute Kidney Injury by Administering Nitric Oxide Gas

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Conflict of interest statement: Francesco Marrazzo and Lorenzo Berra salaries are partially supported by NIH/NHLBI 1 K23 HL128882-01A1. Joseph V. Bonventre is co-inventor on patents that are assigned to Partners Healthcare. Robert M Kacmarek is a consultant for Medtronic and Orange Medical and has received research grants from Medtronic and Venner Medical. None of the other authors has any conflict of interest to disclose.

Author Contributions: Authorship for this trial will be given to key personnel involved in trial design, personnel training, recruitment, data collection, statistical plan, and data analysis. There are no publication restrictions. LB, WMZ, JVB, TBT, NR, EAB, TMS, DF, ER, FM, and FI were responsible for conceptualizing trial design. LB managed patient safety protocol. FM, SS, FZ, CX, GL, and TSL are responsible for recruitment, enrollment, and data collection. RM, HZ, and EAB are responsible for power calculation, statistical plan and data analysis. LB, KS, SM, WDR, PW and RMK trained personnel for the clinical trial and built systems for Nitric Oxide delivery and monitoring. All authors have critically revised the study protocol and approved the final version. All authors agree to be accountable for the accuracy and integrity of all aspects of this trial.

Registration details: NCT02836899

Keywords: Nitric Oxide, Hemolysis, Acute Kidney Injury, Cardiopulmonary Bypass, Endothelial Dysfunction

Word count:

Abstract: 300

Text: 5971

ABSTRACT

Introduction: Postoperative acute kidney injury (AKI) is a common complication in cardiac surgery. Levels of intravascular hemolysis are strongly associated with postoperative AKI and with prolonged (>90 minutes) use of cardiopulmonary bypass (CPB). Ferrous plasma hemoglobin released into the circulation acts as a scavenger of nitric oxide (NO) produced by endothelial cells. Consequently, the vascular bioavailability of NO is reduced, leading to vasoconstriction and impaired renal function. In patients with cardiovascular risk factors the endothelium is dysfunctional and cannot replenish the NO deficit. A previous clinical study in young cardiac surgical patients with rheumatic fever, without evidence of endothelial dysfunction, showed that supplementation of NO gas decreases AKI by converting ferrous plasma hemoglobin to ferric methemoglobin, thus preserving vascular NO. In this current trial we hypothesized that 24-hours administration of NO gas will reduce AKI following CPB in patients with endothelial dysfunction.

Methods: This is a single-center, randomized (1:1) controlled, parallel-arm superiority trial that includes patients with endothelial dysfunction, stable kidney function, and who are undergoing cardiac surgery procedures with an expected CPB duration >90 minutes. After randomization, 80 parts-per-million (ppm) NO (intervention group) or 80 ppm nitrogen (N_2, control group) are added to the gas mixture. Test gases (N_2 or NO) are delivered during CPB and for 24 hours after surgery. The primary study outcome is the occurrence of AKI among study groups. Key secondary outcomes include AKI severity, occurrence of renal replacement therapy, major adverse kidney events at 6 weeks after surgery, and mortality. We are recruiting 250 patients, allowing detection of a 35% AKI relative risk reduction, assuming a two-sided error of 0.05.

Ethics and dissemination: The Partners Human Research Committee approved this trial. Recruitment began in February 2017. Dissemination plans include presentations at scientific

conferences, scientific publications, and advertising flyers and posters at Massachusetts General Hospital.

Article Summary:

Strengths and limitations of this study:

- Supplementation with nitric oxide gas during and after surgery requiring prolonged cardiopulmonary bypass might be most beneficial to those patients with endothelial dysfunction who are unable to replace the hemolysis-mediated NO depletion due to an impaired endothelial nitric oxide synthase (eNOS) activity.
- The number of confounding variables resulting from the liberal use of nephrotoxic agents, the preoperative variability in serum creatinine level and the perioperative optimization of hemodynamics and volume status is minimized, since the “Kidney Disease: Improving Global Outcomes” (KDIGO) guidelines for renal protection are a standard of care at our institution.
- Results from this study could possibly be generalized to other clinical scenarios of intravascular hemolysis and vascular depletion of nitric oxide.

This trial is designed to recruit patients from a single academic institution (Massachusetts General Hospital).

TEXT

Introduction

Acute kidney injury (AKI) is a common and serious complication after cardiac surgery procedures requiring prolonged (>90 minutes) cardiopulmonary bypass (CPB)[1–3]. Because post-cardiac surgical AKI is associated with increased risk of chronic kidney disease (CKD) and mortality[4–6], multiple interventions have been tested in large multicenter trials, but none have been successful at reducing AKI[7–10].

There are two major categories of risk factors for post-surgical AKI. The first is the patient's cardiovascular comorbidities that are associated with impaired endothelial function. These include obesity, diabetes, atherosclerosis, peripheral vascular disease, hyperlipidemia, smoking, and hypertension[2,11]. The second is the extent of CPB-associated hemolysis[12–15]. During hemolysis, plasma hemoglobin (Hb) is released into the circulation, and depletes plasma nitric oxide (NO)[16,17]. NO is a potent mediator of vasodilation and regulates blood flow to tissues[18]. In patients with endothelial dysfunction, the activity of endothelial NO synthase (eNOS), the enzyme responsible for NO production, is impaired and is unable to replenish the NO consumed by plasma Hb[19–21].

In an animal model of free water-induced intravascular hemolysis, Minneci et al. showed that hemolysis resulted in a reduction of creatinine clearance. The negative effects of hemolysis on renal function were attenuated by the inhalation of 80 parts-per-million (ppm) NO gas. NO oxidized plasma Oxy-Hb (Fe^{2+}) to ferric methemoglobin (Met-Hb, Fe^{3+}), thereby decreasing plasma NO consumption[16]**.** In a meta-analysis, the administration of NO gas has been associated with increased risk of AKI in critically-ill patients with ARDS, especially with prolonged use[22]. However, no studies have shown an association between NO administration and increased incidence of AKI in pediatric or adult cardiac surgery population.

In a recent randomized controlled trial, administration of 80ppm NO gas for 24 hours, starting at the onset of CPB, was found to decrease postoperative AKI in a population of young Chinese cardiac surgical patients (average age 48), with no risk factors for endothelial dysfunction, who were undergoing cardiac valve replacement due to rheumatic heart disease[17]. NO administration also improved long-term outcomes such as Major Adverse Kidney Events (MAKE) and reduced stage 3 CKD for up to 1-year after surgery. However, it is still undetermined whether NO gas exerts similar, or even additional, renal protective effects in patients with endothelial dysfunction commonly treated in the United States.

Methods and analysis

Trial design

In this, single-center, randomized (1:1) controlled, parallel-arm superiority trial (RCT), we hypothesized that 24-hour administration of NO gas starting from the initiation of CPB will decrease AKI by converting Oxy-Hb to Met-Hb in patients with signs and symptoms of endothelial dysfunction suggestive of impaired activity of eNOS (primary outcome).

After screening, consented patients who meet the enrollment criteria are randomized to receive either 80ppm NO (intervention group, n=125) or Nitrogen (N_2, control group, n=125) for 24 hours as nitrogen is the major component of air and can be used as placebo. The test gas is administered via the oxygenator during CPB, then by inhalation once mechanical ventilation is resumed. When the patient is extubated, the test gas is delivered by high flow nasal cannula. On the day of surgery, after the insertion of a pulmonary artery catheter (PAC), patients are stratified by preoperative mean pulmonary artery pressure (i.e. mPAP <30 mmHg or mPAP ≥30 mmHg) then allocated into the two study groups. Consented patients with mPAP found to be

≥40 mmHg on the day of surgery after PAC placement, are not randomized in this study because NO or other pulmonary vasodilators might be indicated as part of the medical treatment during the perioperative period. Study outcomes are assessed during the hospital stay, at a 6-week follow-up visit, and by phone at 90 days and 1 year after the surgical procedure.

In order to avoid postoperative AKI, at our institution the "Kidney Disease: Improving Global Outcomes (KDIGO) guidelines" are the standard of care in all cardiac surgical patients[23]. The implementation of these guidelines has been shown to limit the incidence and severity of CPB-associated AKI[24]. Per these guidelines, patients do not receive any nephrotoxic agents such as intravenous contrast agents[25] during the first 48 hours after surgery. Patients receive close monitoring of serum creatinine and hourly urine output, and avoid intraoperative and postoperative hyperglycemia for at least 72 hours after surgery. Hemodynamics are closely monitored to maintain adequate cardiac output (CO) and blood pressure by using intraoperative transesophageal echocardiography (TEE) in combination with the PAC and a radial artery catheter[23,24]. The pulmonary and radial artery catheters are removed only after the patient is discharged from the intensive care unit (ICU) and after completion of test gas treatment.

The study enrollment opened in February 2017 and the first patient entered the trial in June 2017. The study team plans to enroll 250 patients over a period of 3 years. The study design is depicted in Figure 1.

Eligibility criteria

The study population includes men >40 years old and women >50 years old who have signs and symptoms of endothelial dysfunction, have stable kidney function in the 3 months prior to surgery, are undergoing cardiac surgery procedures with expected prolonged CPB (>90

minutes), and are not currently enrolled in other research studies. The presence of endothelial dysfunction is evaluated by using a brief questionnaire that integrates medical history with clinical and laboratory data that has previously been associated with endothelial dysfunction (Figure 2: Screening questionnaire to detect endothelial dysfunction)[26–35]. Eligible patients are those undergoing a scheduled procedure or patients who are already admitted to the hospital. Key exclusion criteria include estimated glomerular filtration rate (eGFR) <30 ml/min/1.73m^2, mPAP ≥40 mmHg and intravenous (I.V.) contrast infusion within 48 hours before surgery. A complete list of enrollment criteria is provided in Table 1.

Intervention

Perioperative management

Perioperative management including the implementation of the KDIGO guidelines has been shown to decrease the incidence of post-cardiac surgical AKI[24]. These guidelines recommend the avoidance of nephrotoxic agents, the close monitoring of serum creatinine and urine output, the avoidance of hyperglycemia, the consideration of alternatives to radiocontrast agents, and a close hemodynamic monitoring to maintain adequate cardiac output, blood pressure and peripheral oxygen delivery. To reduce AKI incidence related to associated factors during prolonged CPB, the KDIGO guidelines have been implemented at our institution as a standard of care in all patients undergoing cardiac surgery.

Anesthesia management of patients enrolled in the study is performed according to the hospital standard of care and using standardized protocols[36]. Before entering the operating room (OR), patients are pre-medicated with 2-5 mg of midazolam. Prophylactic antibiotics (cefazolin 2-3 g or clindamycin 900mg for patients allergic to cefazolin) are administered through a peripheral venous catheter (Introcan Safety® IV Catheter, B Braun). These are given during the hour before the surgical incision. In the OR, electrocardiography (EKG) and peripheral oxygen saturation (SpO_2) are continuously recorded. A radial arterial catheter (Arrow® Seldinger

Arterial Catheter) is placed for continuous blood pressure monitoring and blood sampling. Patients are then pre-oxygenated at an inspired oxygen concentration of 100% for 3 minutes. Anesthesia is then induced with an I.V. bolus injection of 4-6 µg/kg of fentanyl and either propofol 2 mg/kg or etomidate 0.1 mg/kg. Rocuronium 1.2 mg/kg or cisatracurium 0.2 mg/kg are used for paralysis prior to oro-tracheal intubation. Medication doses are adjusted in elderly patients. After intubation, patients are connected to the anesthesia machine, and mechanical ventilation is delivered in volume-controlled mode with a tidal volume of 6 ml/kg. Respiratory rate is titrated to maintain a partial pressure of carbon dioxide ($PaCO_2$) between 35-45 mmHg. The inspired fraction of oxygen (FiO_2) is titrated according to the partial pressure of oxygen (PaO_2), which is measured with arterial blood gas (ABG) analysis. The TEE probe (X7-2t 3D ultrasound probe, Philips) is inserted by the cardiac-anesthesia team for real-time intraoperative feedback to cardiac surgeons on (I) right and left ventricular function, (II) the mechanical stability and the function of repaired/replaced heart valves, and (III) the detection of air emboli/clots. The neck, chest, and abdomen are prepped and draped in a sterile fashion. A urinary catheter is positioned for hourly urinary output monitoring. To determine intraoperative and postoperative effects of NO on hemodynamics, after induction of anesthesia, a PAC (Edwards Lifescience, Irvine, CA) is placed in all patients for continuous hemodynamic monitoring of central venous pressure (CVP), pulmonary artery pressure (PAP), and cardiac index (CI). Electrodes for SEDLine brain function monitoring (Masimo®, Irvine, CA) are positioned on the forehead of each patient to assess the depth of anesthesia by displaying frontal cortex electroencephalogram waveforms and measuring the patient state index (PSI)[37]. The level of anesthesia is maintained by isoflurane administration (0.8-1.2 minimum alveolar concentration) and adjusted based on hemodynamics, while sustaining a PSI score between 30 and 50. Paralysis is maintained throughout the entire procedure by continuous infusion of rocuronium 4-10 µg/kg/min or cisatracurium 1-3 µg/kg/min. When clinically indicated, inotropes or vasopressors are administrated to maintain a mean arterial pressure (MAP) >65 mmHg and a CI >2.2 l/min/m^2.

The perfusion equipment utilized at Massachusetts General Hospital consists of Stockert S5 (LivaNova, Mirandola, Italy) heart-lung machines, CardioQuip (CardioQuip, Bryan, TX, USA) cooler-heaters, the LivaNova Xtra autologous cell salvage system and Medtronic HMS Plus Hepcon system (Medtronic, Minneapolis, MN, USA).

The CPB circuit is primed with 1600 ml Ringer's Lactate and recirculated for priming and air removal. Prior to cannulation, the patient is fully heparinized to a target ACT of no less than 400 seconds and a target heparin concentration of no less than 2.0 I.U./ml. The loading dose of heparin is calculated by the Hepcon device and subsequent ACT and heparin concentration assays are performed on the same system. The cannulation strategy may be central, peripheral or a combination of both depending on the type of surgery (coronary/valve vs. aortic), level of urgency, surgical approach (median sternotomy vs. thoracotomy), patient specific variables such as body habitus, previous cardiac surgical history, vascular disease and vascular anatomic anomalies. Most commonly, an arterial outflow cannula is placed in the distal ascending aorta and venous drainage is achieved by placing a multi-stage venous cannula in the right atrium via the inferior vena cava (IVC). The aortic root is typically cannulated with a catheter to deliver antegrade cardioplegia as well as to decompress the left ventricle. Retrograde catheters are commonly placed in the coronary sinus to maximize myocardial protection during the aortic cross clamp.

Once fully cannulated and prior to CPB, 500ml to 1000ml of prime volume may be removed from the CPB circuit via retrograde autologous priming (RAP) and/or venous antegrade priming (VAP) to reduce the crystalloid burden on the patient upon initiation of CPB. Once the patient is placed on CPB hypothermia is initiated to an extent dictated by type of surgery and surgeon's preference. Mild hypothermia (temperature maintained between 32-35°C) is most common for coronary and valve operations whereas circulatory arrest cases call for deeper hypothermia (temperature maintained between 18-24°C).

Aortic occlusion is achieved by application of an aortic cross clamp and myocardial protection is achieved by administration of either Del Nido cardioplegia[38] or a traditional 4:1 (blood:crystalloid) mixture at hypothermia into the aortic root (antegrade) and the coronary sinus (retrograde). Diastolic arrest is maintained by intermittent doses of cardioplegia every 20-30 minutes.

Blood flow rates while on CPB are maintained at a cardiac index (CI) of 2.4 l/min/m^2 or greater, a MAP of 65-75 mmHg and a urinary output of greater than 0.5 ml/kg/hr. To achieve these MAP goals phenylephrine may be titrated to effect and diuretics such as furosemide (10 mg bolus) may be given. Sweep gas flow and FiO_2 are regulated to maintain a PaO_2 of 150-250 mmHg, $PaCO_2$ of 35-45 mmHg and a mixed venous oxygen saturation (SvO_2) greater than 65%. Arterial and venous blood gases are drawn every 30 minutes and analyzed by the hospital laboratory. Ultrafiltration with a LivaNova hemoconcentrator (LivaNova, Mirandola, Italy) may be performed with or without maintaining zero balance fluid administration for the purposes of normalizing potassium levels (target K^+ 3.5-5.5 mmol/l) and to increase hematocrit (target hematocrit (HCT) greater than 21%), respectively. In the event ultrafiltration is inadequate to increase HCT to greater than 21% packed red blood cells or red blood cells processed by intraoperative autotransfusion may be administered via the LivaNova Xtra autologous cell salvage system. Target blood glucose levels of 100-180 mg/dL are maintained by administration of IV insulin (bolus 10 I.U. and continuous infusion of 2 I.U./hour) when the glucose level exceeds 180 mg/dL.

Once the procedure is complete, the heart and aorta are de-aired and the aortic cross clamp is removed. Temporary epicardial pacing leads are placed, the surgical site is policed for bleeding and the patient is weaned from CPB. Upon satisfactory termination from CPB, the cannulas are removed and the patient is administered protamine sulfate to reverse heparin anticoagulation at a dose indicated by the Hepcon system. Full heparin reversal is confirmed by a heparin level

assay and ACT measurement. Chest tubes are inserted and the sternum is approximated according to procedure and surgeon preference.

After surgery, patients are transferred to the cardiac surgical intensive care unit (CSICU). Standardized protocols for temperature, sedation, pain, glucose, and hemodynamic management are followed. Upon CSICU arrival, active rewarming using a forced-air warming device is performed. Propofol 2 mg/kg/h, ketamine 0.5 mg/kg/h, or dexmedetomidine 0.5-1.5 µg/kg/min are administered and titrated to achieve appropriate sedation. Pain control is achieved with the use of multimodal regimens (I.V. opioids, acetaminophen, and NSAIDS). Within the first 72 hours, blood glucose levels are monitored every 4 hours and I.V. insulin may be used to maintain glucose levels between 100 and 180 mg/dl. Volume status and hemodynamic parameters are continuously monitored throughout the patient CSICU stay by arterial line and PAC. If needed, vasopressors are administered to achieve the following hemodynamic targets: CI >2.2 l/min/m^2, MAP >65 mmHg, and SvO_2 >65%. Cardiac index, pulmonary vascular resistance (PVR), and systemic vascular resistance (SVR) are recorded every 4 hours until test gas suspension. Packed red blood cells (pRBCs) are transfused when Hb <7 g/dl[39]. Urinary output is recorded hourly via urinary catheter. The ICU team may administer furosemide I.V. in presence of oliguria only after optimization of hemodynamic parameters, volume status and assurance of no urinary catheter blockage. No nephrotoxic agents (e.g. I.V. contrast or hydroxyethyl starch products) are administered as recommended by the KDIGO guidelines[23].

Gas delivery

After PAC insertion, patients are randomly allocated to the intervention group (80ppm NO) or the control group (N_2). Using commercially available tanks of N_2 or NO (Airgas Inc, Radnor Township, Pennsylvania) and volumetrically-calibrated flowmeters, pure N_2 (placebo) or 850ppm NO gas in N_2 is mixed with pure O_2 or air. In the NO group, a final concentration of

80ppm NO is obtained. Test gas administration starts at the onset of CPB and lasts for 24 hours. The test gas is administered via the CPB machine into the sweep gas of the CPB oxygenator by regulating the tank as follows:

$$NO\ tank\ flow\ (L/min) = \frac{[sweep\ gas\ flow\ (L/min) \times NO\ concentration\ (80ppm)]}{NO\ tank\ concentration\ (850ppm)}$$

At the end of the CPB, once ventilation is resumed, test gas is delivered via the inspiratory limb of the anesthetic or mechanical ventilator circuit, and thereafter via the mechanical ventilator in the CSICU. The concentration of NO is regulated by using a Medical gas blender. When patients are extubated, they breathe test gas via a facemask or nasal prongs. NO and nitrogen dioxide (NO_2) levels are monitored through in-line NO/NO_2 sensors (Alphasense, Great Notley, Essex, UK). Met-Hb levels and oxygen saturation (SpO_2) are continuously measured using a peripheral pulse co-oximeter (Masimo Rainbow Set™ Technology, Irvine, CA). Schemas illustrating methods of NO and N_2 gas delivery and NO/NO_2 monitoring both during surgery and in the CSICU are reported in Figures 3 and 4.

Abrupt discontinuation of inhaled NO may lead to rebound pulmonary hypertension. This is characterized by worsening oxygenation and increased PAP, resulting in hypoxemia, systemic hypotension, bradycardia, and right ventricular failure[40–42]. For these reasons, after 24 hours, NO is weaned and discontinued while carefully monitoring hemodynamics using the PAC. NO weaning protocols at our institution consists of:

- Decreasing NO to a half dose and maintaining this dose for 5 minutes.
- Further decreasing NO dose to 5ppm and maintaining this dose for 5 minutes.
- Complete discontinuation of NO if no pulmonary hypertension rebound occurs and hemodynamics are stable for 10 minutes after NO gas suspension.

If at any time during the weaning protocol a patient experiences:

- Worsening hypotension (systolic blood pressure, SBP <90 mmHg) requiring the initiation of an I.V. infusion of vasopressor agents or, in patients already receiving vasopressors before NO weaning, a 50% increase of the dose of the vasopressors,
- Worsening hypoxemia requiring an FiO_2 increase of more than 0.2 or a positive end expiratory pressure (PEEP) increase of more than 5 cmH_2O;
- mPAP increase of more than 20% of the value measured before the initiation of the NO weaning, and/or
- A CI decrease below 1.5 $L/min/m^2$ in the presence of a mPAP over 25 mmHg,

a respiratory therapist (RT) resets the NO to the lowest level at which the patient was stable and notifies the principal investigator (P.I.) and the CSICU attending physician.

Failure of NO-weaning is established when one of the above complications occurs.

Blood and urine collection

To assess the extent of hemolysis and the renal-protective properties of NO administration, plasma samples for plasma free Hb, NO consumption, NO metabolites, and serum creatinine measurements are collected immediately before starting the surgical procedure, 15 minutes after the end of CPB, 24 hours after surgery, and 48 hours after surgery[15,17,43–45]. Additional blood samples for serum creatinine measurements are collected once a day for 7 days following the cardiac procedure or until discharge if the patient is discharged before day 7. Finally, a blood sample for serum creatinine is collected 6 weeks after surgery. Five urine samples are collected for urinary biomarkers of kidney injury (immediately before starting the surgical procedure, 15 minutes after the end of CPB, and at 24 hours, 48 hours and 6 weeks after surgery)[46–48]. After centrifugation, all specimens are coded, snap-frozen with liquid nitrogen, and stored on designated shelves in a -80°C freezer.

Outcomes

Primary outcome

The primary endpoint of this therapeutic trial is to determine whether there is a difference in AKI incidence between the control group (receiving N_2) versus the intervention group (receiving NO). AKI is defined by KDIGO criteria as an abrupt (within 48 hours) reduction in kidney function correlated to an absolute increase in serum creatinine of 0.3 mg/dL or more (≥26.5 µmol/L) or a percentage increase in serum creatinine of 50% or more (1.5-fold from baseline) at any time during the first 7 days after surgery or a urinary output <0.5 ml/Kg/h for >6 hours[49].

Secondary outcomes

Secondary renal outcomes include:

1. AKI severity using the KDIGO stages[49]:
    - Stage 1: Serum creatinine increase ≥26.5 µmol/l (≥0.3 mg/dl) or increase to 1.5-2.0-fold from baseline or urinary output <0.5 ml/kg/h for 6 hours;
    - Stage 2: Serum creatinine increase >2.0-3.0-fold from baseline or urinary output <0.5 ml/kg/h for 12 hours;
    - Stage 3: Serum creatinine increase >3.0-fold from baseline or serum creatinine ≥354 µmol/l (≥4.0 mg/dl) or urinary output <0.3 ml/kg/h for 24 hours or anuria for 12 hours or a need for renal replacement therapy (RRT).
2. Requirement for RRT following AKI during hospitalization and at 6 weeks, 90 days and 1 year after surgery.
3. Major Adverse Kidney Events (MAKE) at 6 weeks after surgery. MAKE is a composite outcome of death, new RRT, and worsened renal function (defined as a 25% or greater decline in eGFR)[50].

Secondary non-renal outcomes include:

1. Sequential organ Failure Assessment (SOFA) score during ICU stay[51].

2. Prolonged cardiovascular support defined as the need for vasopressors and inotropic agents, a balloon pump, or a ventricular-assist device for more than 48 hours after cardiac surgery.
3. Maximum hourly vasoactive-inotropic score (VIS) for the first 7 days after surgery and duration of vasopressors and inotropic agents support. VIS is calculated as dopamine dose (mcg/kg/min) + dobutamine dose (mcg/kg/min) + 100 x epinephrine dose (mcg/kg/min) + 10 x milrinone dose (mcg/kg/min) + 10,000 x vasopressin dose (units/kg/min) + 100 x norepinephrine dose (mcg/kg/min) + 10 x phenylephrine dose (mcg/kg/min)[52,53].
4. Duration of mechanical ventilation (hours).
5. ICU and hospital length of stay

Exploratory outcomes

1. Severity of AKI using urinary biomarkers of kidney injury[46–48].
2. AKI incidence and severity related to baseline characteristics of enrolled patients such as the presence of eGFR <60 ml/min at baseline, mPAP at baseline, cardiovascular risk factors associated with endothelial dysfunction, the type of surgical procedure, EuroSCORE II[54]) and intraoperative course (i.e. duration of CPB, duration of aortic cross-clamp, levels of plasma free Hb, levels of NO consumption and NO metabolites) that may impact study results.
3. Delirium assessed using the confusion assessment method for the intensive care unit (CAM-ICU) within the first 7 days after surgery or until ICU discharge[55,56].
4. Quality of life at 6 weeks, 90 days, and 1 year after surgery assessed using the Katz Index of Independence in Activities of Daily Living (ADL) and the PROMIS global health questionnaire[57–59].

Safety outcomes

1. Intra-hospital mortality and mortality at 6 weeks, 90 days, and 1 year after surgery.
2. Non-fatal stroke during hospitalization and at 6 weeks after surgery.
3. Perioperative and non-perioperative non-fatal myocardial infarction[60].
4. Postoperative bleeding calculated as the sum of blood loss through thoracic drains from the moment of chest closure over a period of 24 hours.
5. Need for blood transfusions or transfusions with blood products
6. Postoperative infections (e.g., pneumonia, wound infection, endocarditis, central line infection, urinary tract infection, sepsis).
7. Cardiac arrhythmias and other non-cardiac postoperative complications (e.g., hepatobiliary disorders, pneumothorax, pleural effusion, vascular disorders).
8. Patients requiring a decrease of NO delivery due to Met-Hb >5%.

Sample size planning

The sample size needed for this trial is calculated based on the primary endpoint: the reduction of AKI incidence in the NO treated group (intervention group) compared to the N_2 group (control group). In a randomized controlled trial (RCT) conducted at the University of Muenster (Germany), Meersch et al. showed that implementation of a KDIGO CT surgery "bundle" as recommended by the KDIGO guidelines successfully reduced the incidence of CPB-associated AKI from 71.7% to 55.1%[24]. At Massachusetts General Hospital (MGH), the use of the KDIGO guidelines is part of standard care and the CPB-associated AKI rate is similar (55% as observed in a one-year chart review performed by the investigators for surgery requiring CPB >90 minutes [data not shown]). In a prior trial we conducted in China, we found a 22% relative risk reduction in the incidence of AKI and a 42% relative risk reduction of stage 3 CKD at 1 year in the NO treated group[17]. In this study, we estimate a greater reduction in the incidence of AKI (35% relative risk reduction) because we anticipate that an American population with endothelial dysfunction will benefit more from NO treatment. Thus, in the NO group, the incidence of AKI is

expected to decrease from 55% to 35.75%. Using a Fisher's exact test to compare proportions, the sample size needed to detect a difference, assuming a two-sided type I error of 0.05 and a power of 0.8, is 114 patients per group. In order to account for possible dropouts, we have increased our sample size by 10%. The enrollment plan is 250 patients (125 patients in the NO group and 125 patients in the N_2 group).

Recruitment

This study is performed at MGH in Boston, MA. All inpatients and oupatients undergoing a scheduled procedure are screened for eligibility. If a patient is excluded, the reasons are noted on a screening log. To obtain consent from scheduled patients, the details of the study are given during the preoperative visit at the Cardiac Surgery Preoperative Clinic, along with a flyer overviewing the trial protocol and a copy of the consent form. For patients admitted to the hospital, study details are given at least a day prior to their cardiac procedures. On the day of surgery, prior to the initiation of any study procedures, the patient's written consent is obtained by a licensed physician who is also a member of the study staff. Consent is obtained exclusively from the patient, and not from a surrogate. Patients who choose not to participate in this study receive standard care according to the surgical procedure. After consent is obtained, a de-identified code is assigned to the patient and registered on a dedicated enrollment log.

Randomization and treatment allocation

To ensure balance between study groups with respect to the likelihood of receiving NO after surgery, after the insertion of a PAC, the patients are stratified by preoperative mPAP (i.e. mPAP <30 mmHg or mPAP ≥30 mmHg) then allocated into the two study groups. The randomization sequence is created by an independent statistician using Stata Statistical Software: Release 14 (College Station, StataCorp LP, TX, USA) and then uploaded on the MGH Research Electronic Data Capture (REDCap) application[61]. A predetermined block

randomization method (fixed block size of 10) is used to ensure equal distribution of participants to treatment arms. A physician with no clinical involvement in providing care for the enrolled patients will conduct the randomization procedure throughout the study by using the randomization function provided by REDCap. Thus, the randomization is conducted without any influence from clinicians providing care for the patients, or from statisticians or outcome assessors.

Blinding

The NO and N_2 tanks and the gas delivery systems in the OR and at the bedside are masked and cannot be distinguished on the basis of appearance. This allows to keep participants, clinicians, and investigators blind to the assignment group. For safety and gas monitoring, the clinician administering the test gas remains unblinded to the treatment. This unblinded clinician is solely responsible for gas tank preparation and test gas delivery and monitoring. Assessors who are blind to the treatment allocation conduct assessment of study outcomes. Blinding is maintained until 1 year follow up. The allocation of patients enrolled in the study will not be disclosed before completion of the study (250 patients enrolled). To maintain the overall quality, legitimacy, and integrity of the clinical trial, unblinding of the test gas may occur only in critical circumstances when the responding physician prescribes initiation of NO. In this circumstance, the P.I. fully documents and explains the reasons for unblinding in a report to the Institutional Review Board (IRB).

Data analysis

All trial outcomes will be evaluated using an intention-to-treat analysis plan. Hypothesis testing will be performed using a two-sided significance level (type I error) of α=0.05. Sensitivity analyses per group of treatment will also be performed and compared to the intention-to-treat analysis results. The incidence of AKI (primary endpoint) and postoperative adverse events

after prolonged CPB will be reported in terms of relative risk with 95% confidence interval (CI) in the treatment versus the control group. The median differences (NO group versus control group) with 95% CI will describe differences in continuous perioperative characteristics and continuous postoperative outcomes.

An unadjusted analysis of the primary endpoint will be performed by means of Fisher's exact test comparing incidence of AKI in the NO group against the control (placebo) group. Multivariate logistic regression will be performed to determine if treatment with NO reduces incidence of AKI after adjusting for eGFR, age, sex, and EUROscore II.

Secondary and exploratory endpoints will be addressed using appropriate tests for each outcome. Categorical variables will be compared between the two groups using Fisher's exact test or Chi square as appropriate. Continuous variables will be compared between the two groups using student's t-test or a Mann-Whitney U test as appropriate. The change in the laboratory markers over time and between the two groups will be tested with a mixed effects modeling. Differences among the groups at each timepoint will be tested using an unpaired student's t-test or a Mann-Whitney U test as appropriate.

Interim analysis

An interim analysis by a Data Safety Monitoring Board (DSMB) is planned upon reaching 50% of the study population. The DSMB is comprised of three independent, multidisciplinary experts (online supplementary material: Data and Safety Monitoring Board section). An independent statistician will perform the interim analysis. The statistician will report the results of the data analyzed to the DSMB in a closed session. The DSMB will have access to data collected during the hospital stay and during the follow up visits, including maintenance of patient confidentiality throughout the study. The DSMB may recommend terminating the study if at the interim analysis a significant increase of intra-hospital mortality, mortality at 6 weeks, AKI or need for RRT in either group (N_2 or NO) is detected.

Data management

All data collected for the trial are entered into the MGH Research Electronic Data Capture (REDCap) application[61]. Preoperative, intraoperative, and postoperative data are prospectively collected from the patient's chart until discharge. Follow-up data are collected by interviewing patients at 6 weeks, 90 days and 1 year after surgery.

Data access is restricted and granted by the P.I. only to authorized members of the study team. The P.I. also assigns specific privileges to members of the study team for data import/export. Quality and integrity of the data collected are optimized by using software properties such as logic checks and validation of data fields (i.e. reference range, valid or invalid values). Warnings and error messages alert the co-investigator of missing data or data entries that do not match the requirements set up in the data-entry field. Also, the co-investigators manually perform weekly checks to ensure data consistency. During these periodical checks, all errors detected by the study team related to data collection and data entry are summarized in a data query report. The report is sent to the P.I. following the enrollment of every 5 patients. It is the responsibility of the P.I. to check the original forms for inconsistency, make corrections by modifying the original forms when necessary, and enter a response to the query. Strict confidentiality is maintained by the research team at all times. All forms are and will be kept in a secure, locked cabinet with limited access for at least 5 years after study completion.

Trial management

Study team physicians are responsible for screening all inpatients and outpatients undergoing a scheduled procedure. The number of eligible, consented, enrolled, and randomized patients is recorded in addition to the reasons for non-participation in the trial.

During their hospital stay, patients are closely monitored and all outcomes are recorded. For this reason, no missing endpoints are expected. After discharge, all patients have a scheduled follow-up visit with the cardiac surgeon 6 weeks after surgery. The day before their appointment,

the patient is contacted by a member of the study staff to remind them that a member of the team will collect research data during their visit. This is done by a phone call (or e-mail if unreachable by phone). At the end of the visit with the cardiac surgeon, blood and urine samples are collected for kidney function assessment and a questionnaire regarding medical and surgical complications and quality of life is given. Patients are then called at 90 days and 1 year after surgery to evaluate mid-term and long-term outcomes. To avoid missing follow-ups, a minimum of three calls are attempted by the study team. Calls are made at different times and dates in an attempt to increase the probability of contacting patients. Phone numbers provided by the patient on the screening day are used. If the study team is still unable to contact the patient despite the several phone call attempts, a letter is sent to the home address provided by the patient at their initial screening visit. If all attempts do not provide contact with the patient, a member of the study team may call to the patient's primary care physician or another healthcare provider to obtain information regarding the patient's condition (e.g. deceased). Based on trials previously performed in the Department of Surgery at the MGH, we expect a loss to follow-up of 10%, 15%, and 20% at 6 weeks, 90 days, and 1 year respectively.

Trial risks

Due to the instability of NO, there are risks associated with its use that must be considered. NO reacts slowly with oxygen to form NO_2, which may cause airway inflammation and damage to lung tissues[62]. Moreover, NO oxidizes ferrous Hb to form Met-Hb, which is unable to transport and release oxygen to tissues. However, cyanosis in healthy patients does not appear until Met-Hb levels are 15-20%[63]. The binding of NO to Hb is a rapidly reversible reaction, with a half-life of 15-20 minutes after NO discontinuation. The side effects and adverse events related to NO delivery are well reported in literature. In Table 2 (Summary of Adverse Events from Previous Studies Examining Nitric Oxide), we summarized 8 clinical trials that recorded and reported adverse events associated with NO gas in newborn, pediatric, and adult populations. Based on

the present literature and FDA reports[64], the risks of breathing NO at 80ppm for 24 hours are minimal when Met-Hb levels and NO/NO_2 delivery levels are carefully monitored[65,66]. To improve safety, in the present trial NO is administered and monitored by trained respiratory therapists. The IRB for this study requested monitoring and maintaining levels of NO_2 breathing below 5ppm. We previously tested and found that our delivered circuits do not exceed delivering 1.5ppm of NO_2 when 80ppm of NO gas is delivered at 90% of FiO_2. Met-Hb is continuously monitored by non-invasive co-oximetry. If Met-Hb levels exceed 5% of circulating Hb, the concentration of NO delivered is halved from 80ppm to 40ppm and closely monitored until a reduction occurs. If Met-Hb levels persist above 5%, NO is progressively halved until a reduction below 5% occurs.

Criteria for patient discontinuation

The criteria for patient discontinuation from the study are:

- Voluntary discontinuation by a patient;
- Exiting the protocol for safety reasons based on the judgment of the clinical or research staff. Specific safety reasons include: (1) acute worsening of hypotension defined as a decrease in MAP of >20 mmHg that is not attributable to other causes (such as hypovolemia, hemorrhage or sepsis), but is generally due to worsening of left ventricular failure, (2) sudden worsening of hypoxemia defined as a decrease of SpO_2 to <80% while breathing 100% oxygen and not attributable to other causes (such as pulmonary edema, ARDS, or pulmonary embolism), and (3) an increase in NO_2 levels >5ppm from baseline.

Patient and public involvement

Patients were not directly involved in the study design and recruitment, and did not contribute to the development of the research questions and outcomes. At the end of the trial, upon request,

results will be provided to the study participants and/or their family members and then published on clinicaltrtials.gov.

Enrollment to date

As of May 2019, 161 patients have been enrolled in the study. Assuming that we recruit about 8 patients per month, we anticipate completing the recruitment of 250 patients by May 2020.

Ethics and dissemination

The current trial was approved by the International Review Board (IRB) in August 2016. Further amendments of the study protocol have been performed and clinical trials.gov has been updated accordingly. Analysis of the outcomes of the study (primary, secondary and exploratory outcomes) will be performed when the enrollment of 250 patients is complete. These results will be published in a primary paper, which will be directed to a peer-reviewed journal and may be presented as a poster and/or through oral communication at scientific conferences focused on cardiac surgery, nephrology, anesthesia, and critical care medicine.

Conclusion

This trial is evaluating whether 24-hour administration of NO gas reduces the incidence of AKI after prolonged CPB in patients with signs and symptoms of endothelial dysfunction. The results obtained by this trial may also represent further progress of the scientific community in evaluating the systemic beneficial properties of exposing plasma to NO gas.

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TABLES

Inclusion criteria:

1. Age ≥ 18 years of age
2. Elective cardiac or aortic surgery requiring CPB and with expected CPB > 90 minutes
3. Clinical evidence of endothelial dysfunction assessed by a specifically designed questionnaire (Figure 2)
4. Stable preoperative renal function without evidence of a plasma creatinine increase of ≥ 0.3 mg/dL within 3 months of study entry and without receiving RRT

Exclusion Criteria:

1. eGFR less than 30 ml/min/1.73 m^2
2. Emergent cardiac surgery
3. Life expectancy < 1 year at the time of enrollment
4. mPAP ≥ 40 mmHg and PVR > 4 Wood Units
5. LVEF < 30% by echocardiography obtained within 3 months of enrollment
6. Hemodynamic instability on the day of surgery as defined by a SBP <90 mmHg
7. Administration of one or more pRBC transfusion in the week prior to enrollment
8. X-ray contrast infusion less than 48 hours before surgery
9. Evidence of intravascular or extravascular hemolysis from any other origin:
    - Intravascular: Intrinsic RBC defects leading to hemolytic anemia (eg, enzyme deficiencies, hemoglobinopathies, membrane defects). Extrinsic: liver disease, hypersplenism, infections (eg, bartonella, babesia, malaria), treatment with oxidizing exogenous agents (eg, dapsone, nitrites, aniline dyes), exposure to other hemolytic agents (eg, lead, snake and spider bites), lymphocyte leukemia, autoimmune hemolytic disorders
    - Extravascular: Infection (e.g., clostridial sepsis, severe malaria), paroxysmal cold hemoglobinuria, cold agglutinin disease, paroxysmal nocturnal hemoglobinuria, iv infusion of Rho (D) immune globulin, iv infusion of hypotonic solutions

Table 1. Inclusion and exclusion criteria. CPB: Cardio-pulmonary Bypass; RRT: Renal replacement therapy; eGFR: estimated Glomerular Filtration Rate (CKD-EPI formula)[67]; mPAP: Mean pulmonary artery pressure; PVR: Pulmonary vascular resistance; LVEF: Left ventricular ejection fraction; SBP: Systolic blood pressure; pRBC: packed red blood cells

Authors	Number of Patients	Age Group (newborn, pediatric, adult)	NO (ppm)	Time of Exposure	Adverse Events
Van Meurs et al.[68]	210	Premature newborns <34 weeks gestational age (26 ± 2 gestational weeks)	5-10	10-14 hours	NO_2 >5ppm in 2 infants; Met-Hb >8% in 1 infant. No resulting complications were reported
Stork et al.[69]	114	Newborn >34 gestational weeks and <14 days old (39.3 ± 1.8 gestational weeks)	20-80	<14 days	Met-Hb >5% in 11 infants. No resulting complications were reported. A dose of 100ppm NO was given to two infants for 36 and 60 minutes, respectively. NO_2 levels in the second infant elevated to 5.1ppm. NO_2 and Met-Hb levels decreased after NO concentration returned to 80ppm
Roberts et al.[70]	30	Newborns >37 gestational weeks (39.8 ± 1.5 gestational weeks)	80	<8.5 days	Met-Hb elevated to 18.2% in 1 infant. The later clinical course was uneventful
James et al.[71]	101	Pediatric (1-43 months old)	20	1-3 hours	None related to NO delivery
Cueto et al.[72]	40	Pediatric (15 days old - 17 years old)	4-30	hours to days	Rebound effects of NO withdrawal reported in 2 patients
Lei et al.[17]	117	Adult >18 years old (48.7 ± 9.5 years old)	80	24 hours	None related to NO delivery
Rossaint et al.[73]	9	Adult (17-46 years old)	5-20	3-53 days	None related to NO delivery
Taylor et al.[74]	192	Adult >18 years old (50 ± 17 years old)	5	<28 days	66 reported infections in the NO group and 41 in the control group. Infections deemed unrelated to NO gas administration. No further complications were reported

Table 2. Summary of prior selected studies that reported adverse events related to nitric oxide administration. Data of age are presented as mean ± SD or range.

FIGURES

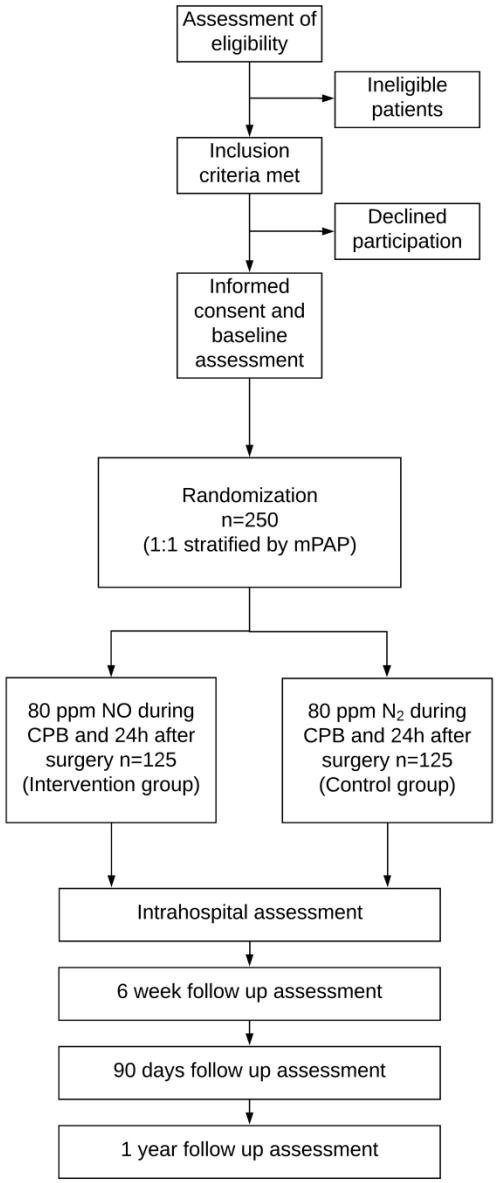
Figure 1. **Study design.** NO: Nitric Oxide; CPB: cardio-pulmonary bypass; N_2: Nitrogen; mPAP: mean pulmonary artery pressure. After placement of pulmonary artery catheter, to ensure balance between study groups with respect to the likelihood of receiving NO after surgery, patients are randomized based on mPAP measured by the pulmonary artery catheter placed on the day of surgery (mPAP <30mmHg and mPAP between 30 mmHg and 39 mmHg).

Figure 2. Screening questionnaire to detect endothelial dysfunction. The questionnaire above aims to systematically detect endothelial dysfunction in patients undergoing a cardiac surgical procedure. If "yes" is answered to at least 1 of the above questions, the patient can be considered to have endothelial dysfunction and he/she may be enrolled in the study. PTCA: Percutaneous transluminal coronary angioplasty; IDDM: Insulin dependent diabetes mellitus; NIDDM: Non-insulin dependent diabetes mellitus; LDL: Low-density lipoprotein; BMI: Body mass index; SBP: Systolic blood pressure;

Figure 3: Schema of the NO delivery systems in the operating room. A. Figure illustrating how the NO or N_2 is delivered into the CPB oxygenator. Tanks of pure N_2 are used in the control group and tanks of 850ppm NO in N_2 are used in the intervention group. A "Y" adaptor is inserted into the sweep gas input line leading to the oxygenator. This allows for the mixing of the test gas with the sweep gas (O2 + medical air). This mixture is periodically monitored with an NO/NO_2 analyzer directly before entering the oxygenator. **B.** Figure illustrating how the NO or N_2 is delivered into the anesthesia ventilator once ventilation has been resumed. The test gas is delivered by placing a "Y" adaptor into the inspiratory limb of the circuit. The mixture is scrubbed of NO_2 by a large volume scavenger containing calcium hydroxide and is periodically analyzed with a NO/NO_2 analyzer before being inhaled by the patient.

Figure 4: Schema of the NO delivery systems in the cardiac surgical intensive care unit.

A. Figure illustrating how the NO or N_2 is delivered through the mechanical ventilator at bedside in the ICU. Tanks of pure N_2 are used in the control group and tanks of 850ppm NO in N_2 are used in the intervention group. Test gas is blended with medical air and enters the air inlet of the ventilator. The high pressure O_2 hose is directly connected to the ventilator. If there is any change of FiO_2, the amount of NO/N_2 delivered is regulated by the RT by adjusting the blender setting and the ventilator FiO_2 setting insuring that the patient is still receiving the target concentration of 80ppm NO. The mixture obtained is then scrubbed of NO_2 through a large volume scavenger and a small volume scavenger placed in series on the inspiratory limb of the circuit. The final amount of NO and NO_2 delivered is periodically analyzed with a NO/NO_2 analyzer directly before the mixture is inhaled by the patient. **B.** Figure illustrating how the NO or N_2 is delivered into the High Flow Nasal Cannula device. The test gas is delivered to the system by placing "Y" adaptor before the humidifier. A commercially available blender mixes O2 and medical air and is regulated by the RT to reach the target FiO_2. The flow of NO_2 or N_2 is titrated to reach the desired concentration 80ppm NO or placebo. This mixture is then humidified and heated to a temperature of 34°C.



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131x295mm (600 x 600 DPI)

CABG and/or valve repair/replacement and age > 40 year old for males and > 50 year old for females and 1 out of 8 of the following criteria:	
Previous coronary artery bypass graft or PTCA (+ stent)	Yes___ No___
History or presence of intermittent claudication, critical limb ischemia, or peripheral vascular disease with the exception of vasculitis.	Yes___ No___
History of transient ischemic attack and/or ischemic stroke	Yes___ No___
Diagnosis of diabetes (IDDM or NIDDM) requiring oral hypoglycemic agents or insulin	Yes___ No___
Hypercholesterolemia (total cholesterol > 200 mg/dl or LDL > 160 mg/dl) treated with statins, ion-exchange resins or other oral agents	Yes___ No___
BMI > 40	Yes___ No___
Hypertension (SBP 140 ≥ mmHg) treated with antihypertensive drugs	Yes___ No___
Active smoking ≥ 10 pack - years	Yes___ No___

Figure 2. Screening questionnaire to detect endothelial dysfunction. The questionnaire above aims to systematically detect endothelial dysfunction in patients undergoing a cardiac surgical procedure. If "yes" is answered to at least 1 of the above questions, the patient can be considered to have endothelial dysfunction and he/she may be enrolled in the study. PTCA: Percutaneous transluminal coronary angioplasty; IDDM: Insulin dependent diabetes mellitus; NIDDM: Non-insulin dependent diabetes mellitus; LDL: Low-density lipoprotein; BMI: Body mass index; SBP: Systolic blood pressure;

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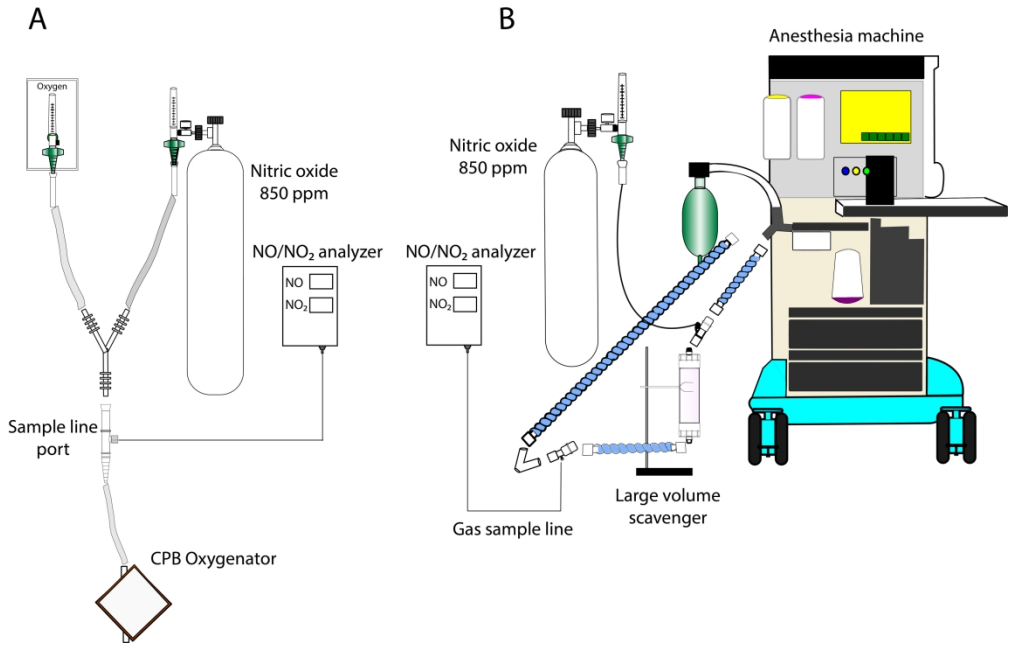

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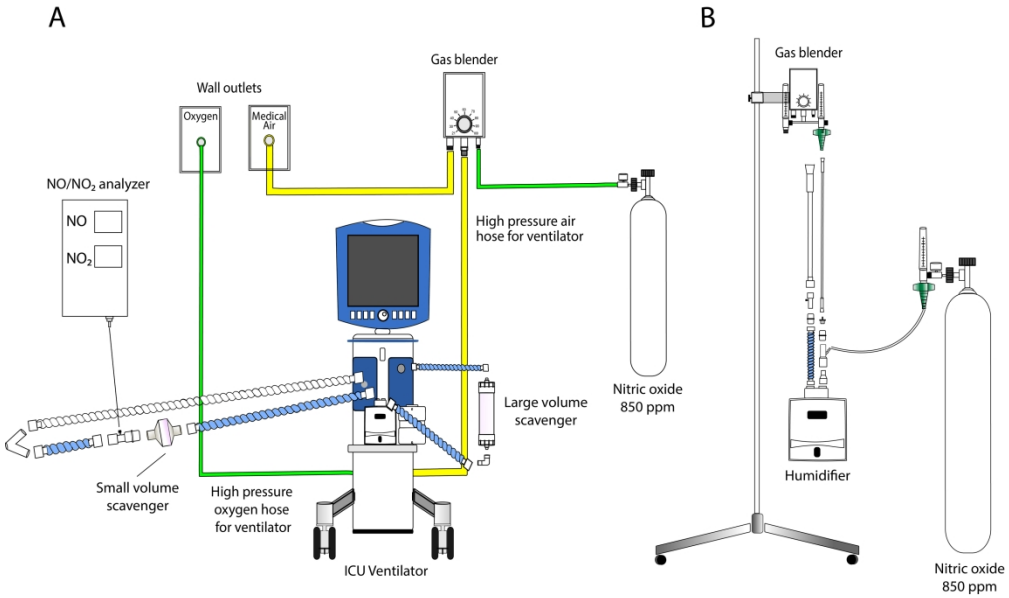

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285x169mm (300 x 300 DPI)

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Primary Registry and Trial Identifying Number	**ClinicalTrials: NCT02836899**
Date of Registration	July 19 2016
Secondary Identifying Numbers	IRB ID#: 2016 P001629
Source(s) of Monetary Support	National Heart, Lung, and Blood Institute (NHLBI) (Award Reference Number K23 HL128882-01A1)
Primary Sponsor	Department of Anesthesiology, Critical care and Pain Medicine Massachusetts General Hospital Boston, MA 02114
Contact name	Lorenzo Berra, MD lberra@mgh.harvard.edu 617-724-2000 Department of Anesthesiology, Critical care and Pain Medicine Massachusetts General Hospital

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Public Title	Effect of Nitric Oxide in Cardiac Surgery Patients With Endothelial Dysfunction
Scientific Title	Prevention of Acute Kidney Injury by Nitric Oxide in Prolonged Cardiopulmonary Bypass: A Double-Blind Controlled Randomized Trial in Cardiac Surgical Subjects with Endothelial Dysfunction
Countries of Recruitment	United States
Health Condition(s) or Problem(s) Studied	Cardiopulmonary bypass associated-Acute Kidney Injury

Intervention(s)	Trial arm 1: Nitric oxide (NO) Group (intervention arm). NO gas 80 parts-per-million is administered via the oxygenator during CPB, then by inhalation when mechanical ventilation is resumed. Once the subject is extubated, NO gas is delivered by high flow nasal cannula.The treatment begins at the onset of the cardiopulmonary bypass and lasts 24h. Trial arm 2: Nitrogen (N_2) Group (Control arm). N_2 gas is administered via the oxygenator during CPB, then by inhalation when mechanical ventilation is resumed. Once the subject is extubated, N_2 gas is delivered by high flow nasal cannula. The N_2 administration begins at the onset of the cardiopulmonary bypass and lasts 24h.
Trial Type	Interventional Allocation: Randomized Intervention model: Parallel assignment Blinding: Clinicians blinded to intervention, participants blinded to intervention, study

	investigators blinded to intervention, primary outcome assessor blinded to intervention. For safety and gas monitoring, only the clinician administering the test gas remains unblind to the treatment. This clinician is not part of the anesthesia, ICU, or surgical physician team delivering care. Assignment: Parallel Primary purpose: Prevention
Date of First Enrollment	June, 2017
Target Sample Size	250 patients
Recruitment Status	Recruiting
Key Enrollment Criteria	Inclusion criteria: Age ≥ 18 years of age; Elective cardiac or aortic surgery with expected CPB > 90 minutes; Clinical evidence of endothelial dysfunction assessed by a specifically designed questionnaire; Stable preoperative renal function without evidence of a plasma creatinine increase of ≥ 0.3 mg/dL within 3 months of study entry and without receiving RRT. Key exclusion criteria:

	estimated glomerular filtration rate (eGFR) <30 ml/min/1.73m^2, mPAP ≥40 mmHg and intravenous (I.V.) contrast infusion within 48 hours before surgery.
Primary Outcome(s)	Outcome name: Incidence of Acute Kidney Injury (AKI) Method of measurement: KDIGO criteria Time points of interest: 7 days after surgery
Key Secondary Outcomes	Outcome name: AKI severity Method of measurement: KDIGO stages Time points of interest: 7 days after cardiac surgery Outcome name: Renal Replacement Therapy Method of measurement: Medical record review Time points of interest: Up to 1 year. Outcome name: Major Adverse Kidney Events (MAKE) Method of measurement: Medical record

review

Time points of interest: 6 weeks after cardiac surgery.

Outcome name: Organ dysfunction (SOFA score)

Method of measurement: Medical record review

Time points of interest: ICU stay (up to seven days)

Outcome name: Prolonged cardiovascular support

Method of measurement: Medical record review

Time points of interest: 48 hours after cardiac surgery

Outcome name: duration of mechanical ventilation

Method of measurement: Medical record review

Time points of interest: up to 6 weeks

Outcome name: Intensive care unit length of stay (ICU-LOS)

Method of measurement: Medical record review

Time points of interest: up to 6 weeks

Outcome name: Hospital length of stay (LOS)

Method of measurement: Medical record review

Time points of interest: up to 1 year

Data and Safety Monitoring Board:

DSMB Chair:

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Responsibilities include: reviewing and evaluating the trial data to ensure participant safety, trial conduct, progress, and efficacy, and making recommendations regarding the continuation, or termination of the trial.

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

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

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

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

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

Chan A-W, Tetzlaff JM, 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
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	3
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	See note 1
Protocol version	#3	Date and version identifier	2
Funding	#4	Sources and types of financial, material, and other support	2
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	See note 2
Roles and responsibilities:	#5b	Name and contact information for the trial sponsor	See note 3

sponsor contact information			
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	See note 4
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)	See note 5
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	7,8
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	8
Objectives	#7	Specific objectives or hypotheses	8,9
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)	8
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, 19, 20
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)	9, 10, 35, 36
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10-16

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)	25
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	23-24
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	23
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	16-19
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, 24
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	19, 20
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	19, 20
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	20, 21
Allocation concealment	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed	20,21

mechanism		envelopes), describing any steps to conceal the sequence until interventions are assigned	
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	20, 21
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	21
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	21
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	22-24
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	22-24
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	22-24
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	21,22
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	21,22
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)	21,22

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	22 and suppl 16
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	22
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	21, 24, 25
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	22, 23
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	21, 26
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)	26
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	20
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	See note 6
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	22-24
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	2

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	22, 23
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	See note 7
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	26
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	See note 8
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	See note 9
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	16

Author notes

1. Suppl material: 9-15
2. Page 1, 3 and suppl material page 1-9
3. Suppl material: 9
4. N/A No role of the study sponsors and funders.
5. Suppl material: 16
6. To participate in this trial, one consent is collected. As indicated in question #33: We plan for collection, laboratory evaluation, and storage of biological specimens analysis in the current trial for possible ancillary studies.

7. Insurance coverage for harm from trial participation will be provided according to Partners healthcare policies

8. N/A we did not plan yet.

9. Suppl material: 17-34