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The Effect of Maintaining Physiologic Oxygenation on Oxidative Stress During Cardiac Surgery

The Risks of Oxygen during Cardiac Surgery (ROCS) trial

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We will test the hypothesis that maintenance of physiologic oxygenation during cardiac surgery reduces oxidative stress and postoperative organ injury compared to the hyper-oxygenation. Markers of oxidative stress, including F_2-isoprostanes and isofurans, increase significantly during cardiac surgery and independently predict a 38% increase in the odds of postoperative kidney injury, a devastating complication of surgery.[1] F_2-isoprostanes are also increased during surgery in patients that develop new-onset atrial fibrillation and may be increased in patients that develop postoperative delirium and long-term cognitive dysfunction. The administration of high doses of oxygen increases the production of reactive oxygen species (ROS) *in vitro* and in rodents, and ROS chemically modify and damage DNA, other proteins, and lipids. Hyperoxia may induce organ injury during surgery.

Anesthesiologists have administered supplemental oxygen to avoid hypoxemia in patients for the last 60 years. The development and widespread use of continuous pulse oximetry hemoglobin oxygen saturation (SpO_2) monitors in the 1980's and point-of-care arterial blood gas measurement machines more recently have not affected the practice of administering excess supplemental oxygen during surgery. During cardiac surgery, for example, anesthesiologists typically ventilate patients with 100% oxygen. Ninety-nine percent of the oxygen transported in blood is bound to hemoglobin, and administering oxygen at concentrations higher than those needed to saturate hemoglobin does not significantly increase oxygen content in blood. It does however increase the partial pressure of oxygen in plasma to super-physiologic levels. These super-physiologic levels may increase the production of ROS[2] and increase oxidative stress and its deleterious consequences in surgical patients.

Not until recently have we realized that the administration of excess oxygen might be harmful to patients. Following cardiac arrest and successful cardiopulmonary resuscitation (CPR), for example, hyper-oxygenation is associated with worse neurologic recovery, coma, and a 44% increase in the odds of death per 100 mmHg change in partial pressure of oxygen (PaO_2).[3] This hyperoxia-associated organ injury may be mediated by oxidative stress.[4] We hypothesize that hyper-oxygenation, defined as the administration of oxygen concentrations greater than what is required to maintain arterial hemoglobin saturation, increases oxidative stress and renal dysfunction compared to maintenance of physiologic oxygenation during cardiac surgery. If we find that maintenance of physiologic oxygenation during cardiac surgery attenuates oxidative stress, we may be able to reduce the neurologic and renal morbidity associated with cardiac surgery worldwide.

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1.0 Background

Mammals require oxygen for oxidative phosphorylation. Prolonged deprivation of oxygen (hypoxia) leads to organ injury and death. Anesthesiologists administer supplemental oxygen (fractions of inspired oxygen [FIO_2] > room air [21%]) to patients undergoing surgery to avoid hypoxia. In the majority of patients undergoing surgery, however, supplemental oxygen administration is not required to maintain hemoglobin oxygen SpO_2, and with the development of continuous pulse oximetry, the excess administration of supplemental oxygen may not reduce the incidence of hypoxia. In addition, oxygen administration in excess of that required for oxidative phosphorylation may increase the production of ROS, leading to oxidative stress. Oxidative stress causes direct damage to proteins including DNA and lipids, resulting in organelle autophagy, cellular apoptosis and necrosis, organ injury and dysfunction, and death.

2.0 Rationale and Specific Aims

Acute kidney injury (AKI) is a devastating complication following cardiac surgery. Five hundred thousand people undergo cardiac surgery each year in the U.S.,[5] and AKI complicates recovery in up to 30% of these patients, requiring renal replacement therapy (dialysis) in 1-4%.[6 9] AKI is associated with postoperative arrhythmias, wound infections, and sepsis, and AKI independently predicts a 5-fold increase in death at 30 days.[10 13] We have demonstrated that F_2-isoprostanes, products of arachidonic acid peroxidation that have emerged as the gold standard for the measurement of oxidative stress *in vivo*,[14] increase significantly *during* cardiac surgery and independently predict an increased incidence of AKI.[1] Isofurans are products of arachidonic acid peroxidation formed preferentially over F_2-isoprostanes under conditions of increased oxygen tension,[15] and we have found that isofurans increase to a greater extent than F_2-isoprostanes during cardiac surgery and are associated with AKI.[16] Hyperoxia increases production of ROS *in vitro* and in rodents,[17] and in recent clinical studies hyper-oxygenation during resuscitation from cardiac arrest independently predicted persistent coma and death.[3 4 18] In preliminary studies, we found that cardiac surgery patients are exposed to oxygen concentrations well above those required to maintain hemoglobin oxygen saturation (median arterial partial pressure of oxygen [PaO_2] = 364 mmHg during surgery) and reducing excess oxygen exposure reduces both F_2-isoprostanes and isofurans. Moreover, reducing excess oxygen exposure was associated with less postoperative renal injury.

We hypothesize that physiologic oxygenation during surgery decreases the generation of reactive oxygen species, decreases oxidative damage, and decreases kidney dysfunction following cardiac surgery compared to hyper-oxygenation. To test this hypothesis we propose the following **SPECIFIC AIMS**:

<u>Aim 1</u>: Test the hypothesis that maintaining physiologic oxygenation during anesthesia for cardiac surgery decreases postoperative kidney injury.

We will recruit and randomize 200 cardiac surgery subjects to hyper-oxygenation (FIO_2 = 0.8-1.0, usual care) or physiologic oxygenation (minimum FIO_2 required to saturate arterial hemoglobin 95-97% and maintain a PaO_2 between 80-110 mmHg) during surgery. We will compare postoperative changes in serum creatinine and markers of nephron injury (neutrophil gelatinase-associated lipocalin [NGAL], between subjects randomized to physiologic oxygenation or hyper-oxygenation to test the hypothesis that maintaining physiologic oxygenation during anesthesia for cardiac surgery decreases postoperative kidney injury. To assess tissue oxygenation in the two study groups, we will continuously measure (automatically recorded every 5 seconds) the FIO_2, SpO_2, brain O_2 saturation, and muscle O_2 saturation. In addition, we will measure the PaO_2, cardiac output, and arterial lactate prior to, during, and following surgery but no less frequently than every hour. We will compare these oxygenation metrics between subjects randomized to hyper-oxygenation vs. physiologic oxygenation to assess oxygen delivery and

consumption between groups. The primary endpoints of Aim 1 will be serum creatinine change (median +/- 95% confidence interval (CI)). Secondary endpoints include additional measures of AKI (incidence of AKI [defined using KDIGO criteria], neutrophil gelatinase-associated lipocalin [NGAL], and insulin-like growth factor-binding protein 7 [IGFBP7]/tissue inhibitor of metalloproteinaseses-2 [TIMP-2]), incidence of delirium (measured using CAM-ICU), duration of mechanical ventilation, duration of hospitalization, SpO_2 (median +/- 95% CI and area-under-the-curve (AUC) < 90%), PaO_2 (median +/- 95% CI, AUC > 150 mmHg, and AUC < 70 mmHg), brain and muscle O_2 saturation (median +/- 95% CI and AUC < 80% baseline), and the percent of FIO_2 titrations within protocol (protocol adherence). Safety endpoints will focus on any risk of hypoxia between study groups and will include postoperative serum CK-MB measurements and rates of arrhythmias, reintubation, transient ischemic attack, stroke, and death.

Aim 2: Test the hypothesis that physiologic oxygenation during cardiac surgery reduces the generation of superoxide (measured by electron paramagnetic resonance of CAT-1H and TMH electron spin probes) and systemic oxidative stress (measured by plasma concentrations of F_2-isoprostanes and isofurans).

In subjects studied in the RCT described in Aim 1, we will measure plasma concentrations of F_2-isoprostanes and isofurans sampled prior to anesthetic induction, 30 minutes after initiation of cardiopulmonary bypass or OpCAB grafting, immediately following separation from cardiopulmonary bypass, at ICU admission, 6-hours after ICU admission, and on postoperative days 1 and 2. We will measure superoxide production in whole blood collected prior to anesthetic induction, immediately following separation from cardiopulmonary bypass, and on postoperative day 1. We will compare these measurements of ROS and systemic markers of oxidative stress between subjects randomized to physiologic oxygenation or hyper-oxygenation during surgery to test the direct effect of excess oxygen exposure on oxidative stress during surgery. By comparing these data to measurements of kidney function will also test a putative but unproven mechanism of surgery-induced AKI.

By accomplishing these 2 Aims we will improve our understanding of the mechanisms of perioperative oxidative stress and postoperative morbidity while assessing any damage of hyper-oxygenation during cardiac surgery. If we determine that hyper-oxygenation increases oxidative stress and/or organ injury, subsequent multicenter studies in cardiac and non-cardiac surgery patients will be performed to validate these findings. Ultimately, we could impact the perioperative management of 30 million annual surgical patients, since the vast majority of patients are hyper-oxygenated during surgery.

Effects on Endothelial Function Sub-study

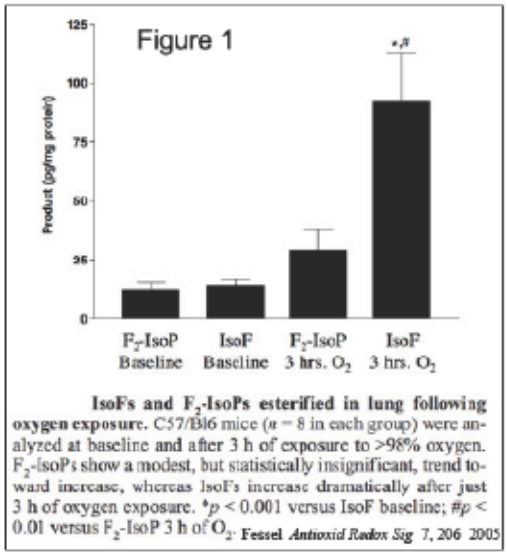
Hyperoxia also induces vasoconstriction,[19 20] and ROS impair endothelial function.[21] For example, pigs exposed to hyperoxia produced less endothelial-derived NO and had increased vasoconstriction compared to controls, and in humans hyperoxia-induced vasoconstriction is prevented by antioxidant treatment, indicating that the vasoconstriction effect of hyperoxia is at least partially mediated by ROS.[22 23] These effects impair tissue perfusion and may be integral to development of postoperative organ injury. Therefore, endothelial function secondary to hyperoxia, secondary to oxidative stress, or independent from oxidative stress may be an important contributor to kidney, brain, and heart injury following cardiac surgery. The ROCS trial provides an ideal framework to test these hypotheses based on the ROCS trial population for the study, randomized oxygen treatments, detailed hemodynamic, perfusion, and oxygenation characterization, blood and tissue sampling, and outcomes assessments.

To test the hypotheses that physiologic oxygenation during cardiac surgery improves endothelial function compared to hyper-oxygenation (sub-study Aim 1), that endothelial dysfunction correlates with oxidative stress in patients having cardiac surgery (sub-study Aim 2), and that perioperative endothelial dysfunction is associated with organ injury (sub-study Aim 3) we will measure endothelial function at baseline (pre-intervention) and following cardiac surgery and compare these measurements between randomized oxygenation groups and to the ROS, F_2-isoprostane/isofuran, oxygenation, and outcomes data generated in the ROCS trial. We will assess endothelial function at baseline and following surgery by quantifying brachial artery flow mediated dilation (FMD), performing peripheral arterial tonometry to

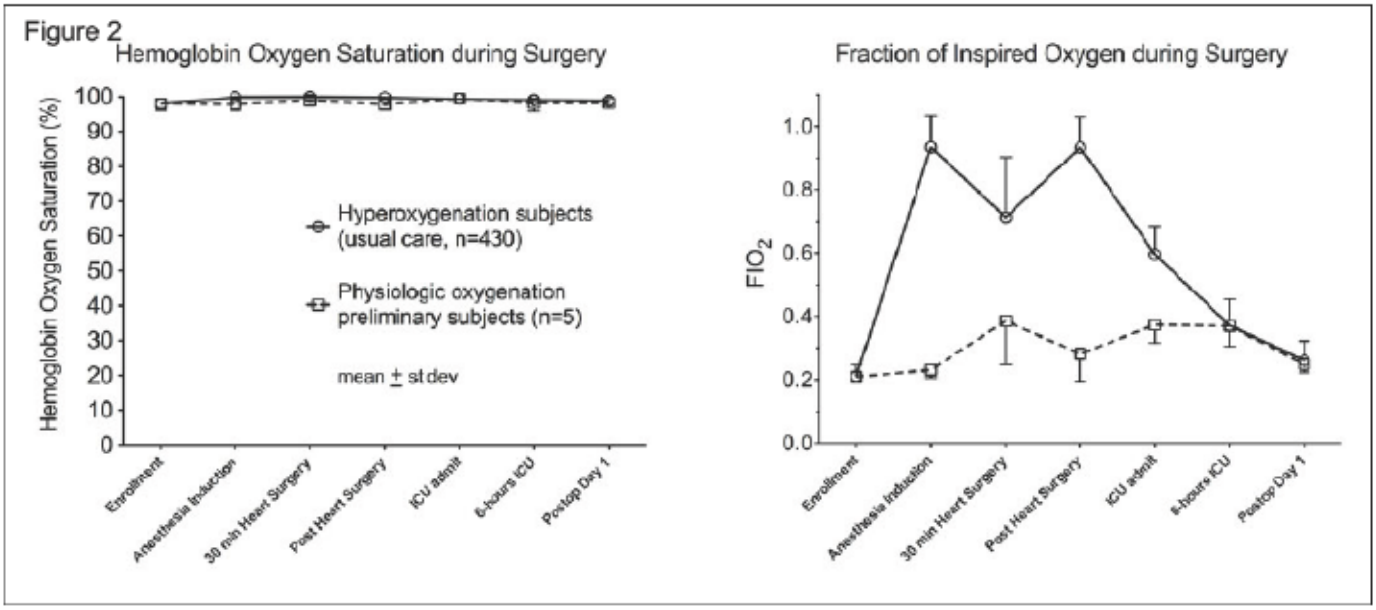
determine reactive hyperemia index, measuring endothelium-dependent and independent vasodilation in epicardial fat arterioles and distal mammary artery segments *ex vivo* using wire myography, and measuring markers of endothelial activation (E-selectin and plasminogen activator inhibitor [PAI]-1) in blood.

3.0 Animal Studies and Previous Human Studies

Oxygen administration increases the production of F_2-isoprostanes and isofurans *in* vitro and in mice (**Figure 1**).[2] In 435 patients from the Statin AKI Cardiac Surgery RCT we recorded oxygen administration (FIO_2) and SpO_2 prior to anesthetic induction (baseline), at induction of anesthesia, during cardiopulmonary bypass or off-pump coronary artery bypass grafting, following cardiopulmonary bypass or off-pump coronary artery bypass grafting, at ICU admission, 6-hours after ICU admission, and on postoperative day 1. In five of these subjects we maintained physiologic tissue oxygenation during surgery by limiting supplemental oxygen administration to that required to maintain SpO_2 > 94% and a PaO_2 > 70 mmHg (**Figure 2**). By doing so, both hyperoxia and hypoxia were avoided.

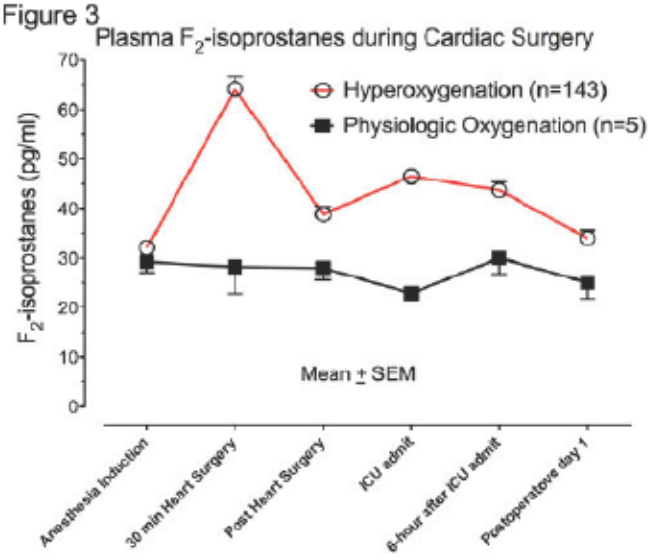


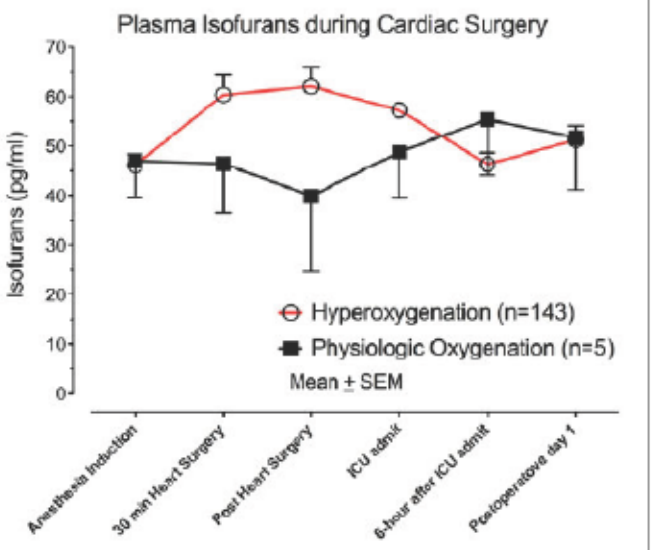
IsoFs and F_2-IsoPs esterified in lung following oxygen exposure. C57/Bl6 mice (n = 8 in each group) were analyzed at baseline and after 3 h of exposure to >98% oxygen. F_2-IsoPs show a modest, but statistically insignificant, trend toward increase, whereas IsoFs increase dramatically after just 3 h of oxygen exposure. *$p < 0.001$ versus IsoF baseline; #$p < 0.01$ versus F_2-IsoP 3 h of O_2. Fessel *Antioxid Redox Sig* 7, 206 2005



Oxidative stress, as measured by plasma concentrations of F_2-isoprostanes and isofurans, was lower in the subjects administered physiologic oxygen concentrations compared to subjects who received hyperoxygenation, matched for risk factors for increased oxidative stress including smoking, body mass index (BMI), and use of cardiopulmonary bypass (**Figure 3**). In addition, none of these physiologic oxidation preliminary subjects suffered from AKI or delirium following surgery, and duration of hospitalization was 6.3 ± 1.6 (median 5.5) days compared to 8.4 ± 4.4 (median 7) in risk-matched controls (P<0.001).

Figure 3





4.0 Inclusion/Exclusion Criteria

Inclusion Criteria:

- Age ≥ 18 years.
- Open-heart cardiac surgery, defined as surgery on the heart or aorta that requires sternotomy or thoracotomy.

Exclusion Criteria:

- Current acute coronary syndrome (defined as ST elevation myocardial infarction or non-ST elevation myocardial infarction (troponin leak within 72 hours of surgery +/- EKG changes consistent with myocardial ischemia)).
- Home supplemental oxygen use.
- Preoperative supplemental oxygen requirement to maintain SpO_2 of 92%.
- Right to left intracardiac shunt including atrial septal defect and ventricular septal defect with Cor Pulmonale.
- Carotid stenosis defined as >50% stenosis.
- Cardiac surgery that requires intraoperative circulatory arrest, such as aortic arch replacement.
- Current use of hemo- or peritoneal dialysis.
- Pregnancy

5.0 Enrollment/Randomization/Blinding

Patients scheduled for open-heart surgery will be screened via StarPanel. Research personnel will request an introduction from the subject's physician. If the subject agrees to the introduction, research personnel will meet with eligible study candidates during their preoperative surgical and / or anesthesia clinic appointment or on the hospital ward. Following explanation of the protocol and documented

informed consent, patients will be randomized to 1) usual care (administration of 100% FIO_2 during surgery and 80% inspired oxygen during cardiopulmonary bypass) or 2) maintenance of physiologic oxygenation (the minimum FIO_2 required to maintain SpO_2 of 95%, but no less than that of air [21%]) during surgery. The intervention is restricted to intraoperative patient management, and upon admission to the ICU after surgery all patients will be ventilated with 40% FIO_2 or the minimum required to maintain SpO_2 95% if greater than 40%. Regardless of treatment group, all subjects will receive 100% oxygen during induction of anesthesia until tracheal intubation and lung ventilation are verified and during transport from the operating room to the ICU. Patients, laboratory technicians, statisticians and ICU staff but not anesthesiologists, perfusionists, and respiratory therapists will be blinded to treatment group. Preoperative, intraoperative, and postoperative care will remain unaffected by inclusion in this study or by study group assignment aside from administration and monitoring of oxygenation.

The study's statistician will be responsible for centralized randomization and subject assignment based on stratification in a permuted block fashion, using a sequence of blocks with sizes either two or four, where the sizes are selected uniformly at random. Randomization will be stratified by chronic kidney disease (defined as stage 3, 4, or 5 CKD [i.e., an eGFR <60 ml/min/1.73m^2]) and use of cardiopulmonary bypass during surgery.

Study anesthesiologists and perfusionists will be responsible for protocol-directed administration of hyper- or physiologic oxygenation. Therefore, anesthesiologists and perfusionists will be aware of subject treatment assignment. We do not expect knowledge of treatment assignment by anesthesiologists or perfusionists to affect other aspects of intraoperative management, including medication or fluid administration. We expect subjects randomized to physiologic oxygenation or hyper-oxygenation to behave the same (similar vital signs, similar responses to surgery, similar response to medications, etc.) and to not warrant any differences in care based on treatment assignment. Operating room surgeons will not be informed of subject assignment, but we will not initiate any procedures such as concealing oxygen and air flow meters to actively blind them from assignment. We do not expect any knowledge of subject treatment assignment by surgeons to affect their treatment of subjects. Study subjects, ICU nurses, intensivists, laboratory technicians, and statisticians will be blinded to treatment assignment throughout subject study. To ensure blinding of ICU intensivists and staff, anesthesiologists will withhold treatment group assignment at the time of subject transfer of care at the end of surgery. Objective endpoints such as laboratory values will be measured by technicians and co-investigators blinded to treatment assignment. Clinical endpoints such as wound infection and delirium will be measured by physicians and research staff blinded to treatment assignment. The key that lists the treatment of each subject will be withheld from investigators, laboratory technicians, and statisticians until the dataset is locked. All preoperative, intraoperative, and postoperative clinical care will be administered according to standardized clinical protocol and will not be affected by inclusion in this study or by study group assignment.

6.0 Study Procedures

We will study 200 adult cardiac surgery patients and randomize them to either hyper-oxygenation during or physiologic oxygenation during cardiac surgery.

Once we have obtained informed consent, we will collect baseline data from the medical record. Upon arrival in the operating room, subjects will be placed on American Society of Anesthesiologists universal monitors as per standard care.

Oxygen administration and monitoring during and following surgery:

1. Hyper-oxygenation control group (usual care):

- Subjects will be preoxygenated with 100% FIO_2 prior to induction of anesthesia.
- Following induction of anesthesia and verification of tracheal intubation and lung ventilation, subjects will be ventilated with 100% oxygen with a tidal volume of 8 ml/kg ideal body weight, respiratory rate titrated to an end-tidal partial pressure of CO_2 of 35 cm H_2O, and positive end-expiratory pressure of 5 mmHg.
- During cardiopulmonary bypass, the oxygenator in the cardiopulmonary bypass circuit will be ventilated with 80% oxygen and gas flow titrated to achieve an arterial blood partial pressure of CO_2 of 50 mmHg. FIO_2 during cardiopulmonary bypass may be increased (see "Titration of FIO_2 during surgery" below).
- During transport from the operating room to the intensive care unit (ICU) following surgery, subjects will be connected to an Ambu bag and mechanically ventilated with 6 liters oxygen per minute fresh gas flow.
- Upon arrival in the ICU, subjects will be placed on the ICU ventilator and ventilated with 40% oxygen with a tidal volume of 8 ml/kg of ideal body weight, respiratory rate of 12 breaths per minute, and positive end-expiratory pressure of 8 mmHg, as is usual practice.
- Subsequent mechanical ventilation, supplemental oxygen administration, and subject extubation will be at the discretion of the ICU intensivist.

2. Physiologic oxygenation group:
    - Subjects will be preoxygenated with 100% FIO_2 prior to induction of anesthesia (similar to hyper-oxygenated group subjects).
    - Following induction of anesthesia and verification of tracheal intubation and lung ventilation, subjects will be ventilated with 21% oxygen with a tidal volume of 8 ml/kg ideal body weight, respiratory rate titrated to an end-tidal partial pressure of CO_2 of 35 cm H_2O, and positive end-expiratory pressure of 5 mmHg. FIO_2 during lung ventilation may be increased and subsequently decreased in order to maintain SpO_2 between 95-97 % (see "Titration of FIO_2 during surgery" below).
    - During initiation of cardiopulmonary bypass, the oxygenator in the cardiopulmonary bypass circuit will be ventilated with 60% FIO_2 and the gas flow titrated to achieve an arterial blood partial pressure of CO_2 of 50 mmHg. Immediately following achievement of full flow on cardiopulmonary bypass, the FIO_2 will be decreased to achieve a SpO_2 between 95-97%, and an arterial blood gas sample will be measured to titrate the inline co-oximeter. Subsequent FIO_2 titrations during cardiopulmonary bypass will be made using the inline co-oximeter to achieve target PaO_2 80-110 mmHg (see "Titration of FIO_2 during surgery" below).
    - During transport from the operating room to the ICU following surgery, subjects will be connected to an Ambu bag and mechanically ventilated with 6 liters oxygen per minute fresh gas flow (similar to hyper-oxygenated group subjects).
    - Upon arrival in the ICU subjects will be placed on the ICU ventilator and ventilated with 40% oxygen or the same FIO_2 the patient was being ventilated at the completion of surgery (whichever is higher), with a tidal volume of 8 ml/kg ideal body weight, respiratory rate of 12 breaths per minute, and positive end-expiratory pressure of 8 mmHg.
    - Subsequent mechanical ventilation, supplemental oxygen administration, and subject extubation will be at the discretion of the ICU intensivist.

Titration of FIO_2 during surgery (for both the hyper-oxygenation and physiologic oxygenation groups):

Throughout the course of intervention, which runs from post anesthetic induction to transfer of care to the ICU staff, the subject's anesthesiologist will continuously monitor the subject's SpO_2 with every beat of the heart, as is standard care. During cardiopulmonary bypass the subject's perfusionists will continuously monitor the subject's PaO_2 with real-time inline co-oximetry, as is standard care. Anesthesiologists and perfusionists are intimately familiar with monitoring patient oxygenation and titrating oxygen delivery. The maintenance of perfusion and tissue oxygen delivery is the most basic responsibility of anesthesiologists and perfusionists.

During lung ventilation, if a subject's SpO_2 falls below 95% for 1 minute, the anesthesiologist will increase the FIO_2 by 10%. If the SpO_2 rises to > 97%, the anesthesiologist will decrease the FIO_2 by 10%. If the anesthesiologist determines the subject requires more than a 10% change in FIO_2, the adjustment of the FIO_2 is at the discretion of the anesthesiologist. The anesthesiologist will wait 2 minutes between changes in FIO_2 unless the anesthesiologist determines the subject requires more rapid titration of FIO_2 to maintain target SpO_2 95-97%. A fresh gas flow of 2 liters/minute will be maintained throughout surgery.

During cardiopulmonary bypass, if a subject's arterial oxygen tension falls below 80 mmHg, the perfusionist will increase the FIO_2 by 10%. If the SpO_2 rises above 110 mmHg, the perfusionist will decrease the FIO_2 by 10%. If the anesthesiologist or perfusionist determines the subject requires more than a 10% change in FIO_2, the adjustment of the FIO_2 is at the discretion of the anesthesiologist and perfusionist. The perfusionist will wait 2 minutes between changes in FIO_2 unless the anesthesiologist or perfusionist determines the subject requires more rapid titration of FIO_2 to maintain target PaO_2 80-110 mmHg.

We will evaluate the following endpoints (primary, secondary, and safety endpoints):

1. Postoperative creatinine change from baseline to postoperative day 2 (Aim 1 primary endpoint). Serum creatinine will be measured daily during hospitalization.

2. Additional markers of AKI including incidence of AKI using KDIGO criteria and urinary markers NGAL and IGFBP-7/TEMP-2.[24]

3. Incidence and duration of delirium, measured using the Confusion Assessment Method for the ICU (CAM-ICU) twice daily while in the ICU or for at least 3 days postoperatively.[25]

4. Oxygen administration, tissue oxygenation, and perfusion, specifically SpO_2 (median +/- 95% CI and area-under-the-curve (AUC) < 90%), PaO_2 (median +/- 95% CI, AUC > 150 mmHg, and AUC < 70 mmHg), brain and muscle O_2 saturation (median +/- 95% CI and AUC < 80% baseline), the percent of FIO_2 titrations within protocol (protocol adherence), cardiac output using a PA catheter placed as part of usual care, mixed venous hemoglobin O_2 saturation, and arterial lactate.

5. Oxidative stress. We will measure F_2-isoprostanes/isofurans (Aim 2 primary endpoint) prior to surgery, during surgery, and following surgery.

6. Reactive oxygen species production using electron spin probes and electron paramagnetic resonance and dihydroethidium and HPLC in blood, myocardium, and epicardial fat.

7. Vascular reactivity (Effects on Endothelial Function sub-study). We will measure flow mediated dilation of the brachial artery using ultrasound and peripheral tonometry with wrist or finger probes before and after inflating a blood pressure cuff, and we will measure vasoconstriction and relaxation in blood vessels dissected from epicardial fat and distal mammary artery that is removed and discarded by the surgeon as part of standard cardiac

surgery in a subset of patients chosen at random (n=40). We will quantify markers of endothelial activation in plasma including PAI-1 and E-selectin.

8. Mitochondrial function. We will measure markers of mitochondrial function including superoxide production, mitochondrial DNA content, mitochondrial membrane potential, PGC-1a mRNA expression, and mitochondrial respiration in PBMCs isolated from blood and atrial tissue resected by the surgeon as part of standard cardiac surgery.

9. Other ICU morbidities including the time to extubation, development of arrhythmias including atrial fibrillation, dialysis, pneumonia (defined as a positive sputum culture or postoperative pulmonary infiltrate with systemic signs of infection [temperature >38.6°C or white blood cell count >12.0 × 109/L] and the use of parenteral antibiotics or documentation of the diagnosis by the patient's physician), wound infection (CDC criteria), and ICU length of stay.

10. Pain assessment on postoperative day 5 and at one year, using the Numeric Rating Scale (NRS) 0-10 pain assessment and the short-form McGill Pain Questionnaire.

11. One year follow-up data including assessments of cognitive function (Short Blessed Test), activities of daily living, and depression (Center for Epidemiologic Studies-Depression (CES-D) test will be obtained by phone interview, and we will examine the clinical chart for admissions, events (any renal replacement therapy), and laboratory findings (with particular focus on serum creatinine, eGFR, and urine proteinuria) in this period.

12. Safety endpoints will focus on any risk of hypoxia between study groups and will include postoperative day 1 serum CK-MB measurements (myocardial injury, defined as CK-MB greater than 50 ng/mL [10 times the upper limit of normal] and CK-MB as a continuous endpoint), postoperative myocardial infarction [defined by a new Q-wave on EKG], rates of reintubation, transient ischemic attack [defined as new deficit on neurologic exam that abates within 72 hours], stroke [defined as new deficit on neurologic exam and confirmed with radiological evidence], and death.

Urine, blood, and tissue use:

Following the placement of the arterial line and Foley catheter, 20 mL of blood and 20 mL of urine will be collected at the induction of anesthesia (baseline). We will also collect 15 mL blood and 20 mL of urine during cardiopulmonary bypass or off-pump coronary artery bypass for those subjects not requiring cardiopulmonary bypass, following cardiopulmonary bypass or off-pump coronary artery bypass, at ICU admission, 6 hours after ICU admission, and on postoperative day 1 and day 2. In total we will collect 110 mL of blood and 140 mL urine. When, after dissection of the pericardial fat, mammary artery, and incision of the atria as part of standard cardiac surgery, 1-2 g of redundant atrial tissue, 1-2 grams of discarded fat, and 1-2 g of discarded distal mammary artery will be collected for measurement of atrial myocardial function, inflammation, amyloid, oxidative stress, and arteriole vascular reactivity. Typically, this tissue is discarded, but instead we will harvest this tissue at the end of surgery. We used the same technique for collection of tissue in 450 cardiac surgery subjects in the Atrial Fibrillation and Renin Angiotensin Aldosterone System (RAAS) study (PI: Nancy J. Brown).[26]

We will also collect data regarding vital signs, electroencephalographic activity, medication use, fluid input and urine output, clinical labs, and postoperative outcomes.

Sample Time-course:

- Preoperative: The PI or his research nurse will offer study participation to adult patients

undergoing cardiac surgery after patients express interest to their surgeon in conjunction with a patient's preoperative surgical consultation appointment, preanesthetic evaluation appointment, or on the hospital ward. We successfully recruited subjects in these venues for 653 patients enrolled in the Statin AKI Cardiac Surgery RCT (PI: F. T. Billings IV).[27] After obtaining informed consent, patients will undergo a brief neuropsychological assessment that will include the Mini Mental Status test, the Trails B test, the CES-D Test, and the NRS 0-10 pain assessment. Patient demographics and past medical history will be documented.

- Day of surgery: anesthesia, surgery, and ICU admission will proceed irrespective of the subject's participation in the study except for the oxygen administration and monitoring and sample collection described above. Study intervention will occur in the operating room and will terminate when subject care is transferred from the anesthesiologist to the intensivist following admission to the ICU.
- Postoperative: subject postoperative ICU and hospital ward stay will proceed irrespective of the subjects' participation in the study and will be controlled by the subject's surgeon, intensivist, and consultants. Patients will be evaluated twice daily while in the ICU or for the first 3 days following surgery for level of sedation using the Richmond Agitation-Sedation Scale (RASS)[28] and for delirium using the CAM-ICU. Both of these assessments are standard practice in the ICUs at VUMC. On postoperative day 5 we will assess patients pain using the NRS 0-10 pain scale and the short-form McGill Pain Questionnaire.
- Subject discharge will proceed irrespective of study. In the Statin AKI Cardiac Surgery RCT, median hospital length of stay was 7 days.
- One year following surgery, we will call patients to conduct a phone interview in which we will assess neuropsychological function and activities of daily living including NRS 0-10 pain scale, short-form McGill Pain Questionnaire, the Short Blessed Test, and the CES-D Test. This assessment will take about 15 minutes to complete.

Data and Safety Monitoring Board (DSMB):

The DSMB will provide objective review of the treatment results as they relate to human safety and data quality. [redacted] have agreed to serve on the committee. [redacted], who will serve as chair, is [redacted].

The DSMB will receive report on the progress, adverse events, safety endpoints, and clinical outcomes at each interim analysis from the PI and the statistician. The first planned interim analyses will occur after 20 (11.1%) subjects have been studied and discharged from the hospital following surgery. At this meeting, the DSMB will evaluate subject recruitment, the documentation of subject data, and the collection and reporting of adverse events, serious adverse events, and safety endpoints. The DSMB will also evaluate any study withdrawals, any adverse events, in-hospital all-cause mortality, and safety outcomes (stroke, TIA, or Q-wave myocardial infarction).

The second planned interim analyses will occur after 100 (50%) subjects have been studied and discharged from the hospital following surgery. At this meeting, the DSMB will evaluate any study withdrawals, any adverse events, in-hospital all-cause mortality, safety outcomes (stroke, TIA, or Q-wave myocardial infarction), and a conditional power analysis implemented by the study statistician. The conditional power analysis will assess the likelihood of rejecting the null hypotheses (for the primary endpoints of Aims 1 and 2) at the study conclusion (N=200), assuming that the effect of intervention is as large or larger than the estimated effect at the second interim analysis (N=100). To do so, the study statistician will compare mock-unblinded data (A vs. B, where it remains unknown if A or B is normoxia or

hyperoxia). If the conditional power is less than the original target (80%), the DSMB will consider recommending an increase in the study sample size to achieve the target power. If an increased sample size is determined to be reasonable by the investigators within the study constraints including feasibility and funding, we will seek approval from the VU IRB to increase the sample size. If the sample size is increased based on the conditional power analysis, an additional interim analysis will be scheduled to occur after 200 subjects have been studied and discharged from the hospital after surgery. At this interim analysis meeting (N=200), the DSMB will evaluate safety by analyzing any study withdrawals, any adverse events, in-hospital all-cause mortality, safety outcomes (stroke, TIA, or Q-wave myocardial infarction), and the unblinded treatment effect on the primary endpoint of Aim 1 (previously only assessed in a mock-unblinded manner for the purpose of conditional power analysis). This analysis will be completed to assess safety not efficacy of the intervention. However, if the sample size is increased, then the statistical significance thresholds for the final analyses will be adjusted to preserve the overall type-I error rate at 5%. For example, if the sample size is increased to 300, then the p-value threshold at the final analysis (for the primary aim using the Wilcoxon rank sum test) will be 0.0499 to preserve the overall type-I error probability at 5%.

There will be no possibility of ending the trial early due to futility or efficacy. All investigators and research personnel (other than the study statistician at the second interim analysis) will remain blinded to treatment assignment during all interim analyses.

We have recruited an experienced and versatile DSMB to help us carefully monitor the safety and efficacy of subject treatment. At either planned interim analysis the DSMB may recommend to continue enrollment and subject study or suspend enrollment and subject study due to safety concerns secondary to study intervention including: increased in-hospital all-cause mortality in subjects randomized to physiologic oxygenation (normoxia) such that the DSMB deems the increase is excessive compared to usual care hyperoxic oxygenation (hyperoxia), increased SAEs deemed “Probably Related” or “Possibly Related” to study intervention in physiologic oxygenation (normoxia) group compared to usual care hyperoxic oxygenation (hyperoxia) group deemed excessive, or increased incidence of stroke, TIA, or Q-wave myocardial infarction in the physiologic oxygenation (normoxia) group compared to usual care hyperoxic oxygenation (hyperoxia) group deemed excessive. The DSMB will require strong statistical evidence, corresponding to a p-value threshold of 0.001, to end the trial early due to safety concerns. The DSMB will not make any recommendations to end the study early due to efficacy or futility.

7.0 Risks

Maintaining physiologic oxygenation during surgery may increase the risk of hypoxemia (SpO_2 < 90%) compared to delivering hyper-oxygenation. Prolonged and severe hypoxemia (SpO_2 < 60% for > 3 minutes) may cause cellular injury, organ infarct, coma, and death. To address any risk of hypoxemia the subject’s SpO_2 will be monitored continuously throughout study intervention by an anesthesiologist and/or certified nurse anesthetist. This monitoring is not unique to this study but is current standard of care in North American operating rooms and includes a visual percent readout of the SpO_2 continuously displayed on two large LED monitors in the operating room (one monitor dedicated to the anesthesiologist and one to the operating room surgeon and staff), automated recording of the subject’s SpO_2 in the anesthetic record every minute during the subjects stay in the operating room, and an auditory note that sounds with every beat of the heart whose pitch reflects SpO_2. If a patient’s SpO_2 decreases below 90%, an audio and visual alarm is activated notifying the anesthesiologist. All of this monitoring is standard practice during the administration of any anesthetic at VUMC and has been developed to reduce hypoxemia through multiple layers of safety alarms and checks. All anesthesia staff are thoroughly trained and intimately familiar with oxygenation monitoring during surgery.

8.0 Reporting of Adverse Events or Unanticipated Problems involving Risk to Participants or Others

Adverse events (AE) will be monitored by the PI and his staff. An AE is defined as any untoward medical occurrence in a subject, not necessarily having a causal relationship with the study. A serious AE (SAE) is defined as any untoward medical occurrence that a) results in death, b) is life-threatening, c) requires inpatient hospitalization or prolongation of existing hospitalization, d) results in persistent or significant disability/incapacity, or e) is a congenital anomaly/birth defect. AEs will be graded as a) Mild (no limitation of usual activities), b) Moderate (some limitation), or c) Severe (inability to carry out usual activities) and attributed according to the relationship to the study drug and/or procedures as a) Not related, b) Unlikely Related, c) Possibly Related, d) Probably Related, or e) Definitely Related. AEs will be submitted to the IRB as per IRB regulation. Specifically, any SAE determined to be possibly, probably, or definitely related to study participation will be reported to the data safety monitoring board (DSMB) and the IRB within 10 working days of the PI's notification of the event (as per IRB standards). Summary reports of any previously unreported SAEs will be reported to the IRB annually and to the DSMB at interim analyses and as requested. These reports will contain the number of SAEs, a description of each SAE, the category of each SAE, and the relationship of each SAE to study participation. Appropriate changes will be made to the consent form as required.

Summary Reports will be submitted to the IRB at least annually and will contain a) The number of adverse events and an explanation of how each event was handled, b) The number of complaints and how each complaint was handled, and c) The number of subject withdrawals and an explanation of why the subject withdrew or was withdrawn.

9.0 Study Withdrawal/Discontinuation

Patients that are withdrawn from study participation will cease study-specific oxygen administration and monitoring, and their surgical and postoperative care will proceed as directed by their clinical caregivers.

10.0 Statistical Analysis Plan

The primary objective of the analysis will be to compare the effect of intraoperative oxygenation on oxidative damage and organ injury. The primary endpoint of Aim 1 will be change in serum creatinine from baseline (defined as the most recent creatinine measured prior to surgery) to postoperative day 2. The primary endpoint of Aim 2 will be F_2-isoprostanes/isofurans measured in plasma.

Sample Size Calculations

We calculated the subject sample size based on data from the Vanderbilt Statin AKI Cardiac Surgery RCT (PI: F. T. Billings IV).[27] Data from this study indicate that mean serum creatinine concentrations increase 0.08 $\pm$ 0.37 mg/dl from baseline to postoperative day 2. We have powered the present randomized clinical trial conservatively, particularly since consensus criteria for AKI diagnosis define AKI as a 0.3 mg/dl rise in serum creatinine from baseline to postoperative day 2.[29] With 200 subjects, we will have 80% power to detect a 0.15 $\pm$ 0.375 mg/dl difference in serum creatinine between groups, with a type I error rate of 5%. In addition, with 200 subjects we will have 80% power to detect a 20 $\pm$ 50 pg/ml difference in plasma F_2-isoprostanes between groups, and we observed that physiologic oxygenation was associated with 35 $\pm$ 30 pg/ml less increase in plasma concentrations of F_2-isoprostanes among pilot subjects compared to hyper-oxygenated subjects. To account for a potential dropout rate of 10%, we plan to recruit 222 subjects.

For the endothelial function sub-study, we calculated the sample size based on a prior study of patients administered an oral antioxidant, treatment improved FMD 2.3% more than placebo with a standard deviation of 2.0%.[30] With 80 patients, we will have greater than 80% power to detect a 2%

difference in FMD change from baseline to ICU admission between subjects randomized to hyper-oxygenation vs. physiologic oxygenation with a standard deviation of 3.0%, with a type I error rate of 0.05 (primary analysis sub-study Aim 1). We will have 94% power to detect a correlation of 0.2 or greater between intraoperative F_2-isoprostane concentrations and postoperative FMD (primary analysis sub-study Aim 2) with standard deviation of 20 pg/ml F_2I and 10 for regression errors. We will have greater than 80% power to detect a 2% difference in FMD between patients who do and do not develop postoperative delirium in the ICU, assuming a delirium incidence of 23.4% among all ROCS trial subjects, similar to that in the Statin AKI Cardiac Surgery RCT. Sub-study Aims 1 and 2 will be completed with 80 ROCS trial subjects and sub-study Aim 3 with all 200 patients.

Randomization

The study's statistician will be responsible for centralized randomization and subject assignment based on stratification in a permuted block fashion, using a sequence of blocks with sizes either two or four, where the sizes are selected uniformly at random. Randomization will be stratified by chronic kidney disease (defined as stage 3, 4, or 5 CKD [i.e., an eGFR <60 ml/min/1.73m^2]) and use of cardiopulmonary bypass during surgery. Thus, there will be four randomization strata. We use the modification of diet in renal disease formula to estimate GFR for randomization stratification. This scheme provides ample subject assignment to all strata.

Data Analysis Plan

Standard graphing and screening techniques will be used to evaluate data quality. Data include patient demographics, baseline and intraoperative clinical characteristics, treatment toxicity and clinical outcomes. Summary statistics of study arms for both numerical and categorical variables will be provided to describe the study sample. Continuous variables will be summarized with the 50th (10th, 25th, 75th, 90th) percentiles, mean and standard deviation, and categorical variables will be summarized with the counts and percentages. For outcomes measured at a single time point, comparisons across randomization groups will be implemented using the Fisher's exact test or Pearson chi-square test for categorical variables, and the Wilcoxon rank sum test or Student T-test for quantitative outcomes, as appropriate. The Clopper-Pearson and Wald methods will be used to construct 95% confidence intervals for the between group effects.

Change in serum creatinine from baseline to postoperative day 2 (Aim 1 primary endpoint) will be compared between normoxia and hyperoxia study groups using the Wilkoxin rank sum test. Plasma concentrations of F_2-isoprostanes and isofurans (Aim 2 primary endpoint) will be compared between normoxia and hyperoxia study groups using a mixed effects regression analysis, adjusting for collection time as a categorical variable (i.e., study phase). This method allows for simultaneous evaluation of multiple correlated measurements per subject, and between-subject heterogeneity in the effects of treatment on markers of oxidative stress. Specifically, we will use a random intercept and random 'slope' for treatment allocation, indexed by study subject. Analysis of the effects of treatment on change in creatinine and on oxidative stress will be repeated separately within randomization strata.

We will evaluate treatment effect on additional markers of AKI (AKI incidence using KDIGO criteria, urine concentrations of NGAL and IGFBP-7/TEMP2, use of dialysis), delirium incidence and duration (diagnosed with CAM-ICU), and myocardial damage (quantified by measuring CK-MB on the morning of the day after surgery) as secondary analyses.

To assess effect of treatment on oxygenation and perfusion we will compare SpO_2, PO_2, cerebral oxygenation, muscle oxygenation, mixed venous hemoglobin saturation, cardiac output, and arterial lactate concentrations between normoxia and hyperoxia groups using mixed effects regression analysis as described above for plasma concentrations of F_2-isoprostanes and isofurans.

We will also compare differences in ROS production, mitochondrial function, atrial fibrillation, stroke, pneumonia, wound infection, time-to-extubation, duration of ICU stay, and in-hospital mortality as well as pain scales, depression data, and neurocognitive function between normoxia and hyperoxia groups as secondary endpoints. We will compare safety endpoints myocardial infarction, transient

ischemic attack, and stroke between treatment groups. Similar to the primary analysis, we will implement logistic, proportional odds and linear regression analyses for binary, ordinal or highly skewed, and continuous outcomes, respectively, using a propensity score adjusted approach, if warranted. Potential impactful covariates included in these models will be specific to each outcome (for example, history of chronic obstructive pulmonary dysfunction would be appropriate covariate for time to extubation analysis).

To test the hypothesis (Endothelial function sub-study) that intraoperative physiologic oxygenation improves postoperative endothelial function (sub-study Aim 1) we will compare change from baseline in FMD (primary endpoint of Endothelial function sub-study), reactive hyperemia index, PAI-1, and E-selectin and the amplitude of endothelium-dependent vasodilation randomized subject groups in the first 80 subjects to complete the ROCS trial. The change from baseline in FMD, RHI, PAI-1, and E-selectin and the amplitude of endothelium-dependent vasodilation will be compared between oxygenation groups using Student's t-test or Mann Whitney U test. To test the hypothesis that endothelial dysfunction correlates with increased oxidative stress during cardiac surgery (sub-study Aim 2) we will compare perioperative concentrations of F_2-isoprostanes and isofurans to endothelial function assessments and plasma markers of endothelial activation at ICU admission in the first 80 subjects to complete the ROCS trial using linear regression and adjust for baseline measurements of endothelial function, and confounders such as randomized treatment group, blood transfusion, and duration of CPB. To test the hypothesis that perioperative endothelial dysfunction is associated with organ injury (sub-study Aim 3) we will compare baseline, postoperative, and change from baseline FMD, reactive hyperemia index, PAI-1, and E-selectin and the amplitude of endothelium-dependent vasodilation to markers of AKI, delirium, myocardial injury, and additional secondary endpoints using logistic, linear, or proportional odds regression as appropriate in the total cohort (N=200). We will review, interpret, and report results from sub-study Aims 1 and 2 based on statistical report from Dr. Shotwell but will remain blinded to treatment assignments in these 80 subjects until the entire 200 patient cohort is completed.

Every effort will be made to avoid missing data. Any methods of missing data imputation (e.g. multiple imputation using the chained-equations method) will be used cautiously as sensitivity analyses; and the analysis of results with imputation would be interpreted in the context of corroboration with the analyses without imputation. The primary analysis will be intention-to-treat. Before data unblinding, Dr. Billings will examine any protocol deviations and identify a list of protocol violators. In addition to the intention-to-treat analysis, a secondary per-protocol analysis will be conducted.

Specific inferences on the effects of interest will be made by reporting point estimates, along with 95% confidence intervals. Hypotheses will be tested at a two-sided significance level of α=0.05. This data analysis plan will be carried out using the statistical analysis package R (R Development Core Team).

11.0 Privacy/Confidentiality Issues

A REDCap electronic data collection form will be designed to minimize missing and erroneous values. Expected ranges are pre-specified to prevent errors such as the shifting of decimal points. The program includes a computerized audit trail so that the identity of individuals entering or changing data and, in the case of changes, both original and revised data are saved. Data are backed up daily. Clinical data will be entered by the research nurse. Research laboratory data will be entered by a senior research technician in the laboratory. A unique identification case number will be used to protect the confidentiality of the study participants. The case numbers and participants' names will be included in the protected source database, but only case numbers will be included in any spreadsheet used for the statistical analysis. Before analysis, the senior research fellow will independently and blindly assess all raw data for accuracy and completeness. [redacted], biostatistician, will check for potential outliers and resolve them with the investigators before unblinding the data and performing statistical analyses.

This project will utilize the REDCap platform for data collection and management. Project team members listed as Key Study Personnel with existing StarPanel access rights may also be granted use of

REDCap Dynamic Data Pull (DDP) tools. These tools are designed to enable transfer of relevant study-related data from the Vanderbilt Research Derivative into REDCap.

We will use the Perioperative Data Warehouse (PDW), an IRB-approved research registry, and the Enterprise Data Warehouse (EDW) to extract additional clinical data. Vanderbilt's electronic healthcare records are stored in the EDW and Vanderbilt's Department of Anesthesiology healthcare records are stored in the PDW. The EDW is an electronic repository of clinical and administrative information, which is updated daily. It stores diverse sources of data from scheduling and billing systems, patient registries, nursing documentation, inpatient and outpatient clinical documentation, computerized order entry systems, pharmacy medication administration records, laboratories, radiological studies, and mortality data from the Social Security Administration Death Master File. Clinical data extracted from the PDW and the EDW data may include but are not limited to demographics (i.e., age, gender, ASA physical status, weight, height, BMI), procedures performed and associated process times (i.e. start of procedure, end of procedure, entry into pacu, discharge from pacu), type of anesthesia associated with procedures, preadmission medications, medications administered during hospital admission, laboratory data (i.e. troponin, CK-MB, creatinine, etc), physiologic information (i.e. vital signs, ventilator data, etc), diagnoses, medical history & co-morbidities, patient allergies, clinical indicators (post-operative pain scores, PONV scores, etc.), medical record (StarPanel) notes regarding any rapid response/code calls, ICU transfers, or in-hospital deaths, and clinical outcomes (i.e., hospital length of stay, mortality, ICU admission, unexpected ICU admission, rapid response/code calls, hospital readmissions, ER visits, etc.) and associated dates.

12.0 Follow-up and Record Retention

Study intervention concludes at ICU admission following open-heart surgery. Identifiers linking participants to study data will be maintained securely for six years following completion of study. Non-personal identifiable data will be maintained indefinitely.

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