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PILOT STUDY TO ASSESS EFFECT OF HIGH DOSE ASCORBIC ACID (VITAMIN C) ON INFLAMMATION REDUCTION IN CARDIAC SURGERY PATIENTS

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Protocol

BACKGROUND AND SIGNIFICANCE

Coronary artery bypass grafting (CABG) is the most common procedure performed by cardiac surgeons. More complicated surgeries were enabled by the development of cardiopulmonary bypass (CPB) in the 1930s; CABG procedures increased to peak at over 500,000 in 2000, although numbers have decreased somewhat in the last decade. In 2010 over 150,000 major cardiac procedures involved CABG; 18,008 involved both aortic valve replacement and CABG; 2,378 involved mitral valve replacement and CABG; and 4,635 involved mitral valve repair and CABG.[1] This procedure is costly; in 2010 mean hospital charges for CABG and valve procedures were $124,404 and $171,270 with a mean length of hospital stay of 9 and 11 days and an in-hospital death rate of 1.8% and 3.9%, respectively.[1-3]

Post-operative atrial fibrillation (AF) is the most common adverse event following CABG, experienced in 20-50% of patients; the highest incidence of AF occurs by the third post-operative day. Post-operative AF is associated with overall poorer prognosis [4,5] , resulting 4-fold increase in disabling stroke and cognitive impairment, 3-fold risk of cardiac-related death[6], and increased length of hospital stay.[7] These risks are increased by the changing composition of the patient population over the last 10 years: patients are older and sicker, and are presenting with more complex disease states and co-morbidities, such as severe coronary artery disease (CAD), congestive heart failure (CHF), chronic obstructive pulmonary disease (COPD), diabetes, previous cardiac surgeries with mechanical support device placements, and complex pharmacological histories. A higher prevalence of AF with increased transfusions among CPB patients has also been reported, suggesting a link with increased plasma load of inflammatory markers and mediators from transfused red cells.[7] Reduction of AF by various drugs is moderately effective, but involves either rate control with beta blockers or rate conversion with amiodarone[8,9], after the myocardial damage processes initiating AF have already occurred.

Decreasing the incidence of post-operative AF, and hence the morbidity and mortality of high-risk CABG patients, could be more fruitfully approached by targeting the upstream combined processes of inflammation and coagulation activation induced by the surgical insult and associated ischemia-reperfusion (I/R). During CPB, the heart is subjected to ischemic periods of varying duration. The cardiac tissue at that point is electrically resting and its metabolic demands are accordingly low. However, there is also a severely diminished supply of oxygen and nutrients. This results in slow but direct myocardium cellular damage, which increases as ischemia duration increases. [7,9] However, after reperfusion with oxygenated blood, oxidative stress actually peaks because of accumulated oxidative substrates and cellular depletion of reductive compounds.[9] Cardiac damage related to ischemia-reperfusion will be exacerbated in the presence of co-morbidities, such as diabetes and CAD, which are pathologies with a significant reactive oxygen species (ROS) injury component. High levels of ROS in the myocardium contribute to both electrical and structural remodeling of the cardiac muscle, resulting in the development of AF.[5]

I/R-related oxidative stress and ROS activation contribute to cell damage by modifying protein DNA and phospholipids, resulting in lipid peroxidation and thiol-group oxidation,

which in turn are linked to the induction of inflammatory cascades. Lipids are important small-molecule metabolites that have roles in a wide variety of physiological processes. The lipidome of eukaryotic cells contains thousands of lipid entities that structurally and chemically regulate cell membranes, store energy, or are precursors of bioactive metabolites. [1, 2] Defects in lipid regulation and metabolism are therefore significant contributors to disease pathophysiology. In particular, eicosanoids are lipid mediators linking coagulation and inflammatory pathways; intracellular oxidant species are essential mediators in eicosanoids synthesis pathways. Thus lipidomic analyses are a potential novel tool for the identification of drivers of underlying pathogenesis and new diagnostic biomarkers.[10]

During CABG, total peroxide (TP) and oxidative stress index approximately double[11] Increased post-bypass plasma levels of inflammatory markers such as C-reactive protein (CRP)[6-10], and platelets and leukocyte activation have been observed during CPB.[12] Increases in oxidative stress associated with CPB are correlated with a reduction in total plasma antioxidant capacity.[11] These observations are of interest because an antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by auto-oxidation; hence antioxidants are often reducing agents.

We propose that cell damage induced by oxidative stress and I/R injury could be prevented and/or inhibited by antioxidant supplementation. Specifically, we hypothesize that high-dose intravenous (IV) vitamin C supplementation will ameliorate ROS and therefore damp down upstream inflammatory processes, leading to a reduction of downstream adverse events with demonstrable links to inflammation processes, such as AF. Vitamin C is a viable therapeutic candidate because it is a powerful antioxidant[4, 12] with an excellent safety profile, and an emerging history of reducing inflammatory markers in both critically ill[6, 13, 14] and traumatically injured[13] patients. Scattered trials have indicated possible benefits of vitamin C in reducing incidence of post-CABG AF (see **Literature search**). An antioxidant such as vitamin C acts as a scavenger of intracellular oxidant species; as these are essential mediators in eicosanoids synthesis pathways, it is expected that vitamin C supplementation would decrease the thrombotic potential of eicosanoid-mediated pathways, such as the thromboxane (TBX) production pathway. Impaired cardiac blood flow and clot formation associated with AF contributes to congestive heart failure and stroke; therefore, reduction of thrombotic potential is another essential feature for the proposed therapeutic agent.

The proposed pilot study described below represents a marked advance on previously-reported trials in that (a) study design is directed towards minimization of systemic bias, and (b) we will perform simultaneous comparisons of multivariate inflammatory and coagulation profile changes over the immediate post-operative time period when inflammatory changes are expected to be at a maximum. The novelty of our approach is our systems-level analysis of lipids and their interacting moieties; new technology in our labs allows for rapid quantitative analysis of over 150 lipid mediators in a sample, time and cost

effective manner.

METHODS/DESIGN

LITERATURE SEARCH

A rapid systematic evidence assessment was conducted on three occasions in PUBMED. Search terms, filtered by identifier *clinical trials,* were:

((high dose vitamin C) OR (ascorbic acid)) AND surgery;
((vitamin C) OR (ascorbic acid)) AND (cardiac AND surgery);
(ascorbate OR vitamin C OR ascorbic acid) AND Superoxide AND (Nitric Oxide);

From a total of 285 studies, 20 were retained; the remainder was excluded for lack of relevance or duplication. Studies were archived and analyzed in Review Manager 5.2.[13]

A search of *ClinicalTrials.gov* using the search terms (*ascorbate* OR *vitamin C* OR *ascorbic acid*) AND (*cardiac surgery* OR *cardiovascular*) AND/OR (*IV* OR *intravenous*) resulted in 9 relevant trials (total 90). As of December 17, 2014, five were completed, one terminated, two of unknown status, and one not yet recruiting; no data from these studies were available.

With very few exceptions, most studies were characterized by high risk of bias in six bias domains (selection, performance, detection, attrition, reporting, other).[14] Lack of randomization and blinding, and failure to explicitly define clinical outcomes contributed to these problems. The majority of studies used oral vitamin C supplementation. Oral doses, even those in excess of 200 mg, are associated with little or no clinically significant increases in circulating Vitamin C levels[15], and pharmacokinetics of oral ascorbic acid dosing differs considerably from intravenous dosing.[16] However most assessed studies reported positive effects of vitamin C supplementation. Meta-analysis of these papers indicated a significant overall reduction in both post-operative AF (9 studies; n= 1039 patients; odds ratio 0.37 [95% CI 0.28, 0.50]) and a small reduction in length of hospital stay (6 studies; n= 672 patients; mean difference -1.3 days [95% CI -1.6, -1.0]). A few papers reported measurements of isolated inflammatory and coagulation markers; however, results were equivocal. For example, vitamin C supplementation of CABG patients (usually in combination with various antioxidant cocktails) seemed to attenuate post-operative oxidative stress,[17] myocardial "injury" (defined as a five-fold elevation of cardiac biomarker values and cardiac dysrhythmia),[18] and endothelial dysfunction.[19] In contrast, a study of cardiac surgery patients on or off CPB did not detect statistically significant differences between oral vitamin C and control groups in C-reactive protein (CRP) concentration, white blood cell (WBC) count, or fibrinogen concentration, markers of inflammation and coagulopathy.[20] However the study was underpowered because of early study termination, and data were uncorrected for non-normality, invalidating subsequent statistical analyses. In summary, although suggestive of benefit, current evidence of efficacy of high-dose vitamin C was of low quality with limited and/or irrelevant outcomes, necessitating development of more rigorous clinical trials.

SPECIFIC AIMS

Specific Aim 1: We will test the prediction that patients administered high-dose vitamin C will show clinically-significant reductions in pro-inflammatory and coagulation markers at peak response and at 48 hours relative to normal saline controls. Specifically, we will measure

a) Levels of circulating pro-inflammatory biomarkers profiles (CRP, IL-6, TNF-α), NGAL (in urine), and lipid pro- and anti-inflammatory mediators (in plasma);

b) Levels of coagulation biomarker and platelet function (e.g. fibrinogen, platelet counts, thrombomodulin, R, K, MA, G, angle, LYO)

Specific Aim 2: We will test the prediction that reduced post-surgical inflammatory burden of patients administered high-dose vitamin C will result in clinically significant reductions in hospital-based outcomes (such as length of hospital stay, length of ICU stay.)

STUDY DESIGN

This is a prospective, double-blind, placebo-controlled, randomized pilot trial comparing high-dose intravenous vitamin C to placebo (normal saline) infusions in elective non-emergent cardiac bypass patients. Double blinding will be used at enrollment, randomization, and throughout treatment, sampling, analysis, and data interpretation phases.

The purpose of this pilot study is to demonstrate **performance feasibility** and **proof of concept (PoC)** before initiation of a full-fledged clinical trial. Both financial and personnel constraints limit the total patient enrollment to 24 (12 per arm). Because sample sizes are so small, hypothesis testing cannot be a central feature of this proposal. Instead, success criteria will consist of tangible and measurable performance metrics relating to trial performance and PoC. *Performance success criteria* relate to the logistics and processes involved in the execution of the study itself (e.g. recruitment, retention, study flow, operations, management, obtaining samples, sample processing, documentation, compliance oversight). *Proof of concept* will be determined by large effect size differences (20%) between test and experimental intervention groups in one or more of the key inflammation and coagulation markers (see **OUTCOMES**).

It is unethical to consider running a phase III trial without sufficient data or feasibility information. This pilot trial will be extremely useful to inform the planning and execution of the future large-scale trial, especially with respect to feasibility and sample size calculations. Proof of concept information will be essential for writing major grants, which usually require such preliminary information for assessing scientific validity[21-23]

Dose/route. **Previous studies** describing use of IV vitamin C[24-26] suggests that a total dose of 200mg/kg/day administered as 4 intravenous doses in 50mL D5W every 6 hours per day for 4 days will be sufficient to permit biologically-effective plasma concentrations, and thus are hypothesized to reduce deleterious effects associated with CPB-induced

inflammation. The intervention schedule in our study was based on a general knowledge of inflammation trajectory; inflammation typically peaks during CPB itself then stabilizes within a 24-hour period[27]. **Accordingly, in this study** intravenous Vitamin C will be given as 200mg/kg/day divided over 4 intravenous doses every 6 hours per day for only 2 days perioperative.

Completion of study drug administration: Study drug administration will be stopped when one of the following conditions is met, whichever comes first:

1. Final drug dose at 48 hours
3. Inability to obtain peripheral access after removal of central line.
4. Discharge from the hospital
5. Withdrawal from study
6. Death

ETHICAL OVERSIGHT

Prior to study initiation, this study will be approved by the Virginia Commonwealth University (VCU) Institutional Review Board, and an IND requested from the Food and Drug Administration (FDA). The trial will be registered with *ClinicalTrials.gov*, and the definitive protocol submitted for publication in one of the open-access clinical trial specific journals (e.g. *Trials*). This is an investigator-initiated study, falling under an existing IND held by another investigator who is not acting as PI. In accordance with GCP, monitoring will occur through the use of a monitor not affiliated with this study. This monitor, while employed at the institution conducting the study, has not been affiliated with the development or execution of this study.

STUDY POPULATION

The study population will consist of adult patients scheduled for elective non-emergent cardiac surgery at Virginia Commonwealth University Medical Center, and who have consented to participation in the study.

Inclusion criteria are: (1) adult (≥18 years) patients (2) scheduled for elective non-emergent cardiac valve replacement or repair with cardiopulmonary bypass, and/or multi-vessel CABG surgery.

Exclusion criteria are: (1) known allergy to Vitamin C; (2) pregnant or breastfeeding; (3) prisoner; (4) emergent cardiac surgery patients; (5) scheduled for single CABG procedure; (6) low ejection fraction (< 35%); (7) current autoimmune disease or on immunosuppressant therapy, including prednisone, prednisolone, dexamethasone, mycophenolate, tacrolimus, cyclosporine, or azathioprine; (8) presence of renal calculi, or renal dysfunction (pre-operative creatinine clearance < 40, or serum creatinine greater than 1.8 mg/dl); (9) known bleeding diathesis such as hemophilia, Von Willebrand's disease, or liver failure, or receiving concurrent anticoagulant therapy (such as warfarin, dabigatran or apixaban) for venous or arterial thrombosis; (10) active infection or tumor; (11) prior history of atrial fibrillation (AF); (12) known diagnosis of diabetes mellitus, or preoperative (this surgery) A1C test result ≥ 6.5; (13) diagnosed with diabetic ketoacidosis

(DKA); (14) known coagulopathy prior to surgery (INR > 1.5).

Patient numbers/sample size: This is a feasibility/ proof of concept pilot trial; therefore power calculations are not appropriate. Patient enrollment numbers are limited *a priori* by the funding available for this project and costs inherent to enrollment, randomization and blinding procedures, sampling, and sample processing.

Consent Procedures. All eligible patients will be approached prior to their scheduled surgery by the study coordinator or attending surgeon. A formal Institutional Review Board (IRB)-approved Informed Consent form will be discussed with the patient, and all questions answered prior to obtaining informed patient consent in writing. Informed consent will be documented in the patient medical record, and the clinical research office and each enrolled patient will receive a copy of his/her signed consent form.

INTERVENTION AND CONTROLS

This is a two-arm trial comparing high-dose IV vitamin C to normal saline placebo controls (Fig. 1). Once informed consent is obtained, the time of surgery will be communicated to the VCU investigational pharmacy so that the medication can be appropriately prepared and delivered to the operating room. Prior to preparation, patients will be randomized to receive either control or Vitamin C solution. Randomization will be performed by a computer-generated permuted block design conducted by the investigational pharmacy. Both test and placebo preparations will be prepared by pharmaceutical staff in identical de-identified 50-mL medication bags so as to be indistinguishable by OR/ICU staff and investigators.

Vitamin C will be administered in doses of 200 mg/kg/day group divided into four doses per day at six hour intervals 48 hours. Patients randomized to the control (normal saline) group will receive equivalent fluid volume in identical dispensing bags; total study fluid volumes will be approximately 400 mL over the two-day study period. Blinded interventions will be administered in the OR by the anesthesiologist, and post-operatively by Cardiac Surgery ICU nursing staff and/or Cardiac Stepdown Units. All participating hospital units will be provided with thorough in-servicing. Nurses will be prompted to administer the medication via the patient-specific electronic medical record (EMR).

OUTCOMES

Enrollment Goals: (1) Up to 40 may be enrolled in order to reach completion of 24 out of 24 subjects to allow for withdrawals; (2) complete follow-up and follow-up data for 24/24 (100%) of enrolled subjects; (3) all patients (24/24) to receive study drug according to randomization designation; (4) all patients (24/24) to receive every scheduled dose of the study drug in a blinded manner; (5) all patients (24/24) to have coagulation and biolipid determinations performed for all.

Biomarker outcomes:

Coagulation and inflammation markers: We hypothesize that oxidative stress acquired during CPB and surgery will upregulate coagulation pathways and vitamin C administration will reduce overall expression and/or reduce the duration of the response. Coagulation testing will include RoTEM, fibrinogen, thrombomodulin, and platelet count. Fibrinogen is a liver-produced acute-phase reactant that increases as a result of inflammation. The release of soluble thrombomodulin is associated with increased pro-coagulant response. Thrombomodulin must be expressed on the endothelium to convert thrombin to an anti-coagulant. The RoTEM measures the viscoelastic properties of clot formation and fibrinolysis. Cytokines are soluble proteins acting as cell signalling and regulator molecules to coordinate generalized inflammatory responses. We will measure a few selected pro-inflammatory cytokines (e.g. IL -6, TNF-α) and non-cytokine markers such as C-reactive protein (CRP) and white blood cell counts. NGAL (neutrophil gelatinase-associated lipocalin) is a marker protein that starts to increase early (earlier than creatinine) in acute kidney injury (AKI). AKI is a very common clinical problem in cardiac surgery ICU (~40%). Both NGAL and creatinine will be followed.

Lipidomics: We hypothesize that oxidative stress acquired during CPB and surgery leads to oxidation of both substrate phospholipids and already-generated precursor molecules, such as arachidonic acid (AA). The oxidation of these lipids would deprive the eicosanoid biosynthetic enzymes of necessary precursors and effectively dysregulate the production of lipid mediators important in maintaining platelet function as well as modulating platelet/leukocyte/endothelial cell interplay. We further hypothesize that administration of high dose vitamin C will attenuate or correct these processes. We have preliminary data suggesting a time-dependent conversion of 2 AA phosphatidylcholine (2-AA-PC) to oxidized 2-AA-PC without a significant effect on total 2-AA-PC levels. This effect correlated with a dramatic reduction in the production of TXB_2. We also have data to show that co-treatment of blood with vitamin C and AA attenuates the loss of TXB_2 levels caused by oxidative stress. Thus, we are confident about the relationship between loss of lipid mediators and previously-demonstrated loss of platelet function induced by oxidative stress.

Our targeted lipidomic analysis will examine a specific subset of lipids identified alterations in the arachidonic acid metabolite pathway associated with inflammation and coagulopathy. We will track patient condition before, during, and after CPB by mass spectrometric profiles of selected biolipids with the initial focus on free fatty acids, eicosanoids, 3-PUFA-derived lipid mediators, phospholipid substrates of cPLA2α (including oxidized forms), and isoprostanes, which are well-characterized markers of oxidative stress and redox status. Lipid entities will be quantitatively determined via LC tandem mass spectrometry.[28-30]

Clinical outcomes: Clinical data will be obtained from the patient's chart under direct supervision of the PI by a research coordinator and/or a graduate student listed in the study personnel. Data will include: (1) Demographics (age, race, ethnicity, gender, height, weight); (2) Pertinent medical history (hypertension, congestive heart failure); (3) Surgery data (cardiac surgeon, date and time of surgery, time and details of bypass, total heparin administered, total protamine administered, blood products administered (type, units),

other concomitant medications [times, dosages/amounts]); (4) Surgical complications (e.g. fever, respiratory failure, intubation (Y/N, duration), chest tube drainage amount); (5) Incidence of AF; (6) Hospital outcomes: length of ICU stay, length of step-down and/or floor stay, and patient discharge disposition. Patients will be followed up through hospital discharge or in-hospital death.

POST-RANDOMIZATION TREATMENT

All clinically-indicated treatments (including blood product, medications, and fluid administration) will be given as appropriate per attending physician discretion.

Study–specific blood samples will be obtained by the anesthesiologist or nursing staff, depending on the patient's location at each time point, at four times:

- 1st Draw: Baseline (after anesthesia induction but prior to drug administration)
- 2nd Draw: Approx. 60 min. after protamine sulfate is administered (during CPB),
- 3rd Draw: 24 hours post-baseline (+/- 2 hours),
- 4th Draw: 48 hours post-baseline (+/- 2 hours).

Approximately 20 mL of blood - 2 Na citrate tubes and 1 SST tube - will be needed to run assays described below. In addition, 3 urine samples will be collected, 5 mL each, at Baseline, 6hr, and 24 hrs. Blood and urine tubes will be transported to the analysis research lab by a study personnel member.

DATA STORAGE AND MANAGEMENT

Confidentiality will be maintained by storing paper-based records in a secure central location with access provided only to the study staff. All records will be de-identified and data coded with the key stored in a separate secure location. All personal identifying information will be kept in password protected files and these files will be deleted after 6 years, per VCU requirements. Other de-identified records, such as lab results, will be kept in a locked file cabinet for 6 years after the study ends and will be destroyed at that time. No data will be stored indefinitely.

Patients will be screened, recruited, and consented in a private exam room prior to their scheduled surgery. Collection of identifiable information will only be necessary to link the subject to medical records and/or subject data. Personal information about subjects may be shared with or copied by authorized officials of the Federal Food and Drug Administration and Department of Health and Human Services if required. Although results of this research may be presented at meetings or in publications, no identifiable personal information will be disclosed.

DATA SAFETY MONITORING BOARD (DSMB) AND SAFETY OVERSIGHT

An independent DSMB has been appointed to oversee safety monitoring and trial data comprised of physicians and a biostatistician who are not directly involved with the study and interventions.

PROTOCOL ADHERENCE

Deviations from study protocol will be reported by the Trial Coordinator to the PI and, as needed, to the VCU IRB.

ADVERSE EVENTS AND/OR DRUG REACTIONS

Very few side effects have been observed in clinical trials of intravenous high-dose vitamin C.[14] The only known reported toxicity of Vitamin C originates after continuous (days to weeks) of infusions or intake, resulting in the formation of oxalate renal calculi.[12] Another potential concern is the hypothesized pro-oxidant property of high dose Vitamin C; however, evidence supporting this claim is weak; antioxidant properties at even higher doses were demonstrated to be predominant over pro-oxidant properties.[32] The safety of the proposed dose has been verified in our preliminary human data, and the safety of higher doses has previously been verified in cancer trials.[33] Overall, high-dose IV Vitamin C is regarded as having a good safety profile with a low risk of toxicity over a wide range of doses.

High-dose vitamin C levels can lead to a false reading of blood glucose levels using point of care testing. Blood glucose levels are to be measured in the metabolic lab as detailed below in the Glucose Monitoring Plan section.

GLUCOSE MONITORING PLAN:

Ascorbic acid is known to artefactually **raise** POC blood glucose readings; however, it does not raise blood glucose readings from a basic metabolic panel (BMP). Thus, extreme care must be taken to assure an accurate blood glucose level from a metabolic laboratory before initiating any insulin therapy, including sliding scale or scheduled insulin.

Guidance for blood glucose monitoring in patients enrolled this study:

- Critical care Nursing and Physician leadership at all study sites must be informed of vitamin C's effect on point of care (glucometer) blood glucose and arterial blood gas glucose point of care values.
- In-service training will be documented in the Study Training Log
- Bold signage will be displayed on all study instructions, data collection forms, and at the patient's head of bed, stating:
  - STOP! This patient is enrolled in a study with Vitamin C, which artefactually increases POC glucose testing
  - Do NOT use Accuchek or other Point of Care devices to measure glucose on this patient

- Use ONLY metabolic glucose screening performed by central laboratory as ordered in Cerner

- Do NOT initiate or utilize sliding scale, scheduled insulin, or continuous insulin infusion without laboratory confirmation of blood glucose level

- Those receiving insulin infusion or sliding scale insulin will have metabolic glucose screening on the schedule determined by the primary physician and paid for by the study
- Blood glucose monitoring for insulin administration guidance should *only* be by a metabolic laboratory measured blood glucose results, whether or not the study patient is receiving insulin
- To accommodate the required glucose monitoring via the metabolic lab, each subject will have a dedicated peripheral line
- Study personnel will follow each study patient closely to monitor insulin use to ensure that point of care glucose screening is suspended for the research subject.
- If subject loses central venous access (PICC line and arterial line acceptable), Vitamin C infusions are to stop but subject not withdrawn. Data will be collected through end of study.
- Point of care glucose testing may resume 36 hours after the last infusion of study drug.

ADVERSE EVENT REPORTING/SAFETY REPORTING

Investigators will determine daily if any clinical adverse experiences occur during the period from informed consent through study hour 48 and will be followed up through resolution, resolved with sequelae, unresolvable or death.

It is expected that diseases/illnesses/symptoms associated with the study population will occur in the study population, independent of investigation product exposure. These associated diseases/illnesses/symptoms will be considered as part of the study inclusion processes and/or study assessments and as such will not be considered 'reportable' Adverse Events (AE)/Serious Adverse Events (SAE) unless the Investigator has a reasonable doubt regarding the relatedness of the event to the investigational product.

The investigator will evaluate any changes in laboratory values and physical signs and will determine if the change is clinically important and different from what is expected in the course of treatment of the study patients. If clinically important and unexpected adverse experiences occur, they will be recorded on the adverse event case report form.

The following will be considered reportable adverse events:

For this trial, a *reportable adverse event* is defined as:

1. Any clinically important untoward medical occurrence in a patient receiving study drug or undergoing study procedures which is different from what is expected in the clinical course of a patient undergoing cardiac bypass surgery, or,
2. Any clinically important, untoward medical occurrence that is thought to be associated with the study drug or procedures, regardless of the "expectedness" of the event for the course of a patient undergoing cardiac bypass surgery.
3. Investigators will report all *serious, unexpected, AND study-related* adverse events from the time of informed consent through study hour 48 that are considered to be harmful and unintended responses to the investigational product and/or study related procedures in the participants' case report forms. 'Responses to investigational product' means that the causal relationship between an investigational product and an adverse event cannot be ruled out.

The following will be reported as adverse events:

Investigators will report all unanticipated problems that **involve risk or harm to a research participant** AND **was not anticipated or foreseen (e.g., not described in the consent form)** AND **is probably or definitely related to or caused by the research** to the sponsor/IND Holder by phone and email within 24 hours of becoming aware of event. The Institutional Review Board will be notified within 5 business days of receiving notice of the unanticipated problem.

The study team will report all unanticipated problems, defined as problems that **involve risk or harm to a research participant** AND **was not anticipated or foreseen (e.g., not described in the consent form)** AND **is probably or definitely related to or caused by the research**, to the DSMB within 7 calendar days of the Sponsor/IND Holder being notified of the event.

The DSMB will also determine if the serious adverse event is unexpected for Vitamin C. If the DSMB determines that any serious and study-related adverse event is unexpected for Vitamin C, the FDA will be notified within 7 calendar days. Such events may also meet the definition of *Unanticipated Problems* as described below.

Investigators must report *Unanticipated Problems*, regardless of severity, associated with the study drug or study procedures within 24 hours. An unanticipated problem is defined as follows:

Any incident, experience, or outcome that meets all of the following criteria will be reported from the time of consent through study hour 48 until resolved, withdrawn from the study, death occurs or lost to follow up:

- Unexpected, in terms of nature, severity, or frequency, given the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and the characteristics of the subject population being studied;

- Related or possibly related to participation in the research. In this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research;
- Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

WITHDRAWAL CRITERIA

The Principal Investigator may withdraw the subject at any time due to subject failure to adhere to protocol, or for safety concerns.

TRIAL MANAGEMENT

It is expected that the trial will terminate when the intended sample size (24 patients) has been achieved. However, the trial will be stopped prior to completion if: (1) the intervention is associated with adverse effects that call into question the safety of the test intervention; (2) problems with study recruitment or retention will significantly impact the ability to evaluate the study endpoints; (3) any new information that becomes available during the trial necessitates discontinuation of the trial.

The trial protocol and results will be published in peer-reviewed journals. All publications will follow the Consolidated Standards for Reporting Trials (CONSORT) statement [25]. Credit for the study will be assigned to collaborators who have actually performed designated work. The results of the trial will be reported first to trial collaborators. Summary outcome data and links to published data will be uploaded to the *ClinicalTrials.gov* website.

ETHICAL CONSIDERATIONS

This research will involve the use of fresh blood drawn from patients. The blood draws will be done, when possible, from in-dwelling arterial line and central line catheters. Additional venous blood draws from peripheral sites (e.g. antecubital, radial vein) may be required if the arterial and central line catheters have been removed before the conclusion of 48-hour study period, heparinized, or would be compromised by extra blood draws. Blood draw volumes will be approximately 20 mL.

The toxicity of Vitamin C is very low; therefore, potential benefit far outweighs known risks.

Patients will not be paid for participation and there will be no penalties imposed for either refusal to participate or withdrawal of consent after enrollment.

Patient Drop-Out. Patients may refuse consent and leave the study at any time for any reason. This will be done by notifying the study physician or study coordinator. Subjects can be withdrawn from the study emergently by the attending study physician if they

develop signs and symptoms of adverse reactions.

DATA ANALYSES

Baseline demographic and clinical characteristics of study participants will be reported as descriptive summary statistics. Categorical variables will be reported as absolute and percent values. Continuous variables will be expressed as means and standard deviations, or median and interquartile ranges.

For each primary and secondary outcome, we will report the point estimate of effect and its precision (e.g. 95% confidence interval).

Biomarkers will be analyzed both individually and as clusters, or constellations, of variables. These clusters will be determined by exploratory factor analysis, discriminant analysis and/or vector analysis. Each cluster can be interpreted as a specific pathophysiological network process. The clusters can then be used as an empirical model to determine inter-dependencies between individual biomarkers. Discriminant function analysis is used to predict patient membership in either the Vitamin C treatment group or the control group by the entire suite of inflammation and coagulation variables (the predictor variables). Unlike cluster analysis, discriminant analysis is used when groups are known *a priori*. The measures of effect size are the *canonical correlation* (correlation between groups and the function), the *proportion of variance for each function*, calculated as $(\lambda_x/\Sigma\lambda_i)$ where λ_x is the eigenvalue for the function and $\Sigma\lambda_i$ is the sum of all eigenvalues, and *percent correctly classified*.[34] Finally vector analysis[35] can allow determination of the simultaneous directional change of all the measured inflammatory, biolipid and/or coagulation markers as a function of treatment group. These techniques allow a comparison of dynamic responses rather than a direct comparison of static levels which may not be particularly revealing of complex patterns of interactions between multiple variables.

No interim analyses will be performed because this trial is a small pilot.

LIMITATIONS OF PROPOSED RESEARCH

Small sample size. This is a pilot study designed to assess feasibility and proof of concept; constraints due to restricted funding limited the scope of this initial trial. Because this is a pilot trial, statistical significance cannot and should not be emphasized in determining clinical relevance and significance of findings as this trial is not powered to detect minimal differences between groups.[22]

Missing markers. Preliminary subsets of biomarkers were carefully chosen according to biological relevance, greatest relevance to cardiovascular conditions, and published strong associations with clinical outcomes. Nevertheless, relatively few biomarkers will be screened, so it is possible the relevant features of the inflammatory response may not be captured, and/or sufficient alteration in the inflammatory profile may not occur to allow discrimination between test interventions. However, the sensitivity of TA1 is optimized to the femto-molar range for most analytes. If this is not sufficient to detect much lower

concentrations present at early stages of the post-operative inflammatory response (as indicated by sample analyses and mathematical modeling) we will employ a charge reversal derivatization for detecting sub-low limit of quality level of oxilipins and free fatty acids.[36,37]

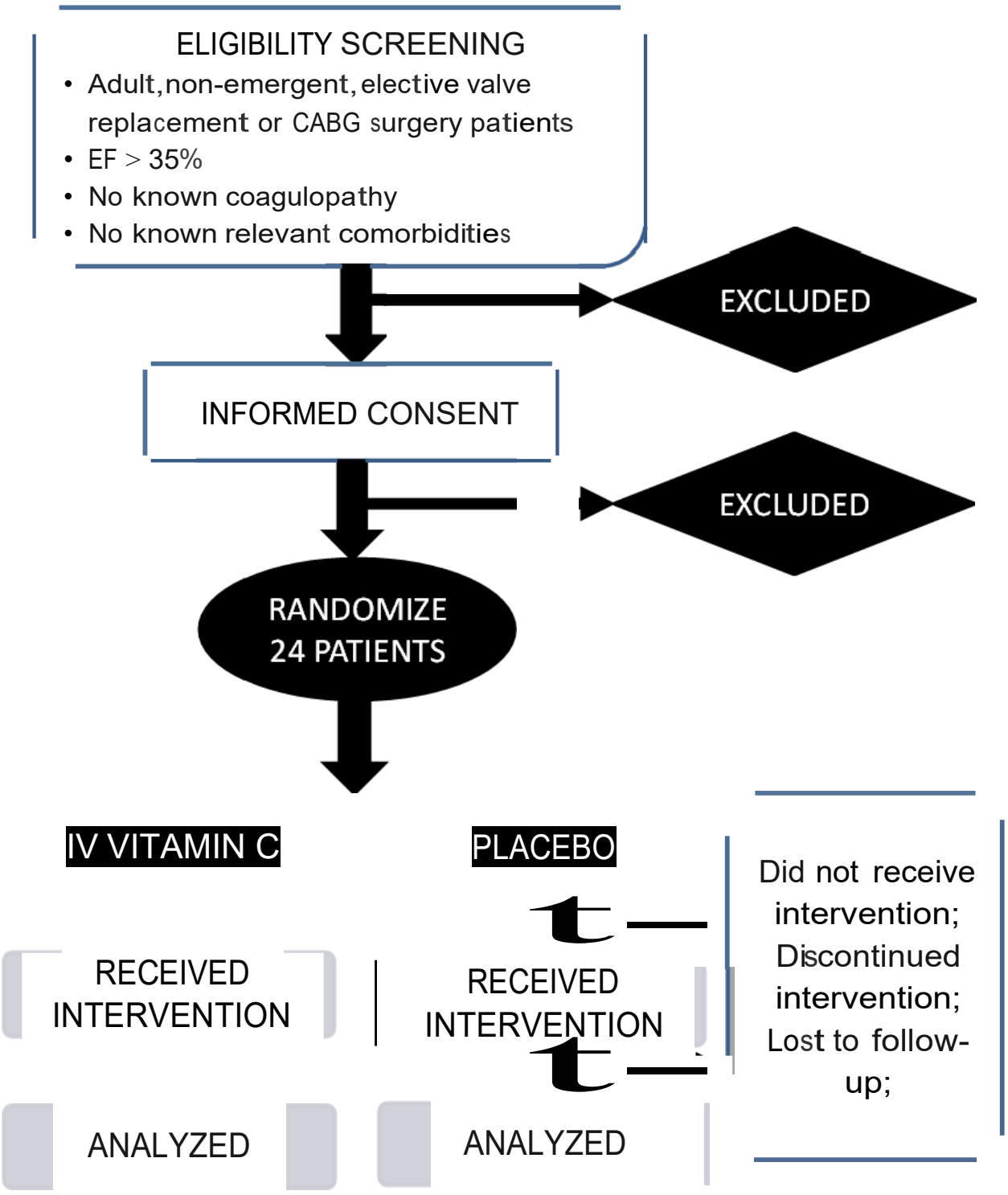
Misidentified markers. Possible sample contamination may result in some non-lipid metabolites being misidentified as important markers. Analytes will be checked for conformity to identification parameters of experimental or theoretical lipid databases.

FUTURE RESEARCH

Pilot study results will be used to determine if proceeding to a large, adequately-powered, main trial is feasible. There are four possible feasibility outcomes:[22]

a) **Stop:** Main study not feasible (because of poor recruitment retention, staffing, insufficient resources, or high probability of treatment futility);
b) **Continue, but modify protocol:** Feasible with modifications (most likely scenario; this could include modifications of operational logistics, dose loads, dosing schedules, data acquisition protocols, etc.);
c) **Continue without modifications, but monitor closely** (for example, if the major limitation is ensuring patients get the appropriate test intervention at the appropriate time, we could have in-place monitor);
d) **Continue without modifications**: feasible as is.

Figure 1. Trial flow per CONSORT guidelines



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