A Pilot Study Involving the Changes in the Markers of Coagulation during Cardiopulmonary Bypass Surgery Utilizing Acute Normovolemic Hemodilution

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Sponsor

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Date of Protocol: 12 April 2019

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PROTOCOL SYNOPSIS

Title: A Pilot Study Involving the Changes in the Markers of Coagulation during Cardiopulmonary Bypass Surgery Utilizing Acute Normovolemic Hemodilution

Short Title: TARGET ANH

Rationale: The results of this study will help enhance current understanding of the impact of Acute Normovolemic Hemodilution (ANH) administration on transfusion requirements post cardiac surgery, the effect of this procedure on coagulation, and platelet hemostasis, and the amount of chest tube drainage 24 hours post coronary artery bypass graft surgery (CABG) surgery.

Objectives:

Primary:

The primary objective will be assessment of hemostasis at baseline and serially during coronary artery bypass graft surgery utilizing the cardiopulmonary bypass machine in patient utilizing acute normovolemic hemodilution.

Study Type: Single center study, prospective, observational, open label.

Study Design: A total of 60 subjects will be included in the study, 40 subjects, between the ages of 18- 85 years old that will undergo first- time isolated (CABG) utilizing cardiopulmonary bypass (CPB) with the administration of ANH, and 20 controls undergoing first- time isolated CABG between the ages of 18- 85 years old not receiving ANH .

Study Methodology: We will analyze the hemostatic markers of 60 patients undergoing cardiac surgery utilizing cardiopulmonary bypass (CPB) pre and post ANH infusion, and without ANH infusion at various time points which shall be different for both the experimental (ANH recipients) and the control group (non-ANH recipients) culminating at 24 hours post CABG surgery to evaluate the need for allogenic blood transfusion, changes in coagulation and platelet hemostatic markers, and effect on 24 hour chest tube drainage. Time points for the control group (non-ANH recipients) will include seven blood draws (7): baseline, 30 minutes post-CPB machine initiation, once immediately off of CPB, and post protamine infusion. Further draws will be obtained at 1 hour post CPB withdraw, 2-4 hours post CPB withdraw, and finally at 16-24 hours post CPB withdraw.

Time points for the experimental group (ANH recipients) will include eight (8) blood draws: baseline, 30 minutes post-CPB machine initiation, once immediately off of CPB, and post protamine infusion and after ANH reinfusion. Further draws will be obtained at 1 hour post ANH reinfusion, 2-4 hours post ANH reinfusion, and finally at 16-24 hours post ANH reinfusion.

Statistical Methodology: Categorical variables will be expressed as n (%). Continuous variables will be reported as mean± (SD)], or median (interquartile range), where appropriate. Paired t-tests or Wilcoxon signed rank tests or will be performed to test differences in lab values before and after treatment. Correlation analysis will be performed by Pearson's or Spearman's Rho, where appropriate. Univariate and multivariate logistic regression models will be used to determine predictors of clinical endpoints (24-hours chest output and total number of transfused

packed blood cells). The predictive cut-point values of platelet function tests to clinical endpoints will be assessed by receiver operator characteristic (ROC) curve analysis with Area Under Curve (AUC) statistics. We will use separate repeated measures generalized linear mixed models to associate changes in hemostatic markers, reduced chest-tube drainage, reduction in transfusion, etc. Statistical calculations will be carried out using SAS (Ver. 9.3, Cary, NC).

1. INTRODUCTION

1.1 Specific Aims

- To better understand the relationship between ANH utilization during CABG and its effect on the quantity and character of specific biomarkers of thrombogenicity at baseline and serially pre- withdraw and post- reinfusion of the patients collected blood.
- To identify variations in thrombogenic phenotypes which play a role in the decreased incidence of bleeding complications among patients undergoing ANH transfusion post CABG and those patients who do not undergo ANH transfusion. And to obtain pilot data to clarify the influence of the ANH procedure on clinical outcomes as evidenced by the incidence of perioperative allogenic blood transfusion and quantification of 24 hour chest tube drainage post CABG.

1.2 Hypothesis

We hypothesize that ANH will be associated with favorable changes in hemostatic markers, a reduced total number of transfused, packed red blood cells, and less chest tube drainage at 24 hours after CABG surgery. We will also gain a better understanding the characteristics of the blood components intraoperatively and postoperatively which are responsible for these favorable outcomes.

1.3 Background and Significance

Cardiac surgery is foremost among procedures that consume allogenic blood transfusions. Excessive bleeding in cardiac surgery is multifactorial in that it can be caused by the surgical procedure itself or the effects of the surgery on hemostasis [1]. And while allogenic blood transfusion has been identified as a major mechanism of harm in cardiac surgery patients it is balanced by the harmful sequelae of bleeding [2]. Predictors for the need of allogenic blood transfusion include; advanced age, preoperative anemia, small body size, female sex, preoperative antithrombotic therapy, coagulation disorders, reoperation, and prolonged CPB time [3]. Preoperative hematocrit is the most frequent indicator of intraoperative transfusion in CABG patients [4]. The incidence of chest tube drainage greater than 1000 ml in the 24 hours post CABG surgery is a strong predictor of mortality in this patient population [2].

The profound risk factors which exist for allogenic blood transfusion in the context of CABG utilizing CPB are; infection, circulatory overload secondary to blood volume increase, immune suppression, transfusion mediated acute lung injury, hospital acquired infection, renal failure, stroke, febrile reactions, hemolytic reactions, severe sepsis, sternal wound infection, post- operative pneumonia, multiple organ failure, frequent admission to intensive care, increased length of hospital stay , hospital mortality, and most significantly an increased short and long term mortality

[4,5,6,7,8,9,10,24]. Patients undergoing CABG are also at risk for anemia related to perioperative bleeding, coagulopathy, hemodilution secondary to pump priming solutions [11, 12, 13] and pump trauma.

The use of allogenic transfusion to increase tissue oxygenation in perioperative anemic patients while theoretically effective is disputed by many studies which demonstrate no increase in tissue oxygenation attributable to allogenic blood transfusion [14, 15]. Hemodilution is a strategy that may avoid or limit the utilization of allogenic blood [16].

Acute Normovolemic Hemodilution (ANH) involves the removal of the patients own blood immediately at the induction of anesthesia and replacement of that blood with an equivalent volume of crystalloid or colloid solution in order to dissuade the loss of RBC [17] cell mass, the mechanism of action is dilution of the blood, hematocrit reduction, and reduction of the anticoagulant components of the blood [18] associated with bleeding during CABG utilizing CPB. The removed blood is isolated from the negative effects of CPB such as the activation and consumption of platelets [19], the retention of RBC's in the pump mechanism, and the destruction of blood products secondary to pump trauma. The collected blood is then stored in anticoagulant treated blood bags and reinfused into the patient in reverse harvest order [16] at the commencement of surgery. This reinfusion method ensures the highest quality blood, that richest in cell mass is administered last [25]. Success of this blood product conservation technique is dependent upon the preoperative high hematocrit count, and volume of blood collected balanced with the amount of intraoperative blood lost [16, 17].

A review of relevant literature identifies many situations in which ANH is beneficial; in patients that refuse blood transfusion for religious reasons ANH provides RBC's, plasma, and platelets, in patients who have a preoperative high hematocrit count [20] ANH eliminates the risk of transfusion reaction inherent with allogenic blood components, preserves erythrocytes from CPB damage, enhances coagulation through the readministration of the patients own clotting factors and platelets, and improves perfusion via reduction of blood viscosity which in turn delivers more oxygen to the tissues by increasing blood flow to the stenosed and collateral circulation in the myocardium [21]. ANH reverses the effects of CPB administered heparin and the reduced blood oxygen carrying capacity secondary to hemodilution [21]. ANH is easy to perform, low in cost, with no evidence of the untoward effects of coagulation, hemolysis, fibrinolysis, or negative immune response in the collected blood as opposed to RBC transfusion [21].

However, the beneficial attributes of ANH are countered by studies that highlight; the time consuming nature of harvesting and reinfusing the patient's blood, an initial low hemoglobin count limits the amount of blood that can be collected, and the role of intraoperative blood conservation has been touted to also reduce the need for allogenic transfusion without the disadvantages of ANH [22] collection and reinfusion. Also confounding the benefits of ANH is the marked disparateness between trials of ANH as a blood conservation strategy, studies with

inadequate power, and frequency of studies not specifically designed to evaluate the efficacy of ANH and therefore devoid of definitive assessment of clinical benefit [17].

In order to avoid the negative sequelae associated with allogenic blood transfusion, utilization of the blood conservation method of ANH will be an effective strategy for avoiding loss of cell mass during on pump CABG surgery. Exactly how this procedure is beneficial, most significantly its effects on platelets and clotting mechanisms is a significant gap in knowledge.

We propose to study the effect in patients undergoing ANH on; the need for allogenic blood transfusion, hemostatic markers, and chest tube drainage 24 hours post CABG. Positive effects in reduced number of transfused RBC's, the hemostatic markers of anticoagulation and platelets, and reduced chest tube drainage at 24 hours post CABG will indicate a positive correlation with ANH utilization during isolated on pump CABG surgery. We propose that this study will provide us with a better understanding of the effects of ANH on coagulation, specifically platelets and how ANH contributes to better outcomes associated with clotting.

1.4 Preliminary Studies

In our earlier analysis of 1,319 non- isolated CABG patients, we compared post- operative morbidity and mortality in patients with and without ANH. We found that ANH was associated with a reduced risk for intraoperative blood product use (OR= 0.41, $p<0.001$), including use of RBC (OR= 0.32, $p<0.001$) and fresh frozen plasma (OR=0.49, $p<0.001$). Transfusion of intraoperative RBC and FFP was independently associated with greater risk for prolonged mechanical ventilation ($p<0.001$ and $p=0.003$, respectively) and operative mortality ($p=0.005$ and $p=0.04$). However, how ANH influences hemostasis in patients undergoing cardiac surgery is unknown [27].

2. STUDY DESIGN AND SUBJECT SELECTION

2.1 Study Type

This is an observational study that will be conducted in subjects undergoing first- time isolated CABG surgery.

2.2 Setting/Location

Inova Heart and Vascular Institute, 3300 Gallows Road, Falls Church, VA 22042

2.3 Duration of Study

Patient participation will entail perioperative phlebotomy during the course of CABG surgery utilizing CPB and postoperatively with the last blood draw completed 16-24 hours after the cessation of CPB. Medical records will be reviewed for adverse events and discharge date upon hospital discharge.

2.4 Number of Subjects

Sixty (60) patients are expected to participate in this trial.

2.5 Study Population

2.5.1 Population Characteristics

2.5.1.1 Gender and racial and ethnic origin of subjects

The study's intended population is inclusive of both genders (males and females), and all racial and ethnic groups and subgroups.

2.5.1.2 Age of subjects

Subject enrollment will be comprised of subjects 18 to 85 years of age.

2.5.2 Vulnerable Population

Children, pregnant women, institutionalized persons, and persons with decisional incapacity will be not be enrolled in this study.

2.6 Recruitment

Recruitment will occur in one of two settings; while inpatient at the IHVI upon first contact with the cardiac surgery service, while hospitalized but prior to the day of surgery in order to give the patient and family time to review the consent, or outpatient at the Inova Cardiac Surgery offices at 2921 Telestar Court, Falls Church, Virginia. The patient will be considered enrolled in the study after signing informed consent. The expected length of the recruitment period is 8 months. Approximately 300 coronary artery bypass graft surgeries are performed at the IHVI each year. If the study conduct (e.g. recruitment rate; drop-out rate; data quality; protocol compliance) does not suggest a proper completion of the trial within the reasonable time frame as agreed upon, the recruitment period may be extended to reach the desired sample size.

2.7 Inclusion Criteria

- Subject is 18 to 85 years old.
- Subject undergoing an isolated CABG procedure with or without ANH
- Subject is hemodynamically stable
- The subject is able to read and has signed and dated the informed consent document including authorization permitting release of personal health information approved by the investigator's Institutional Review Board (IRB).

2.8 Exclusion Criteria: Subjects will be excluded from entry if ANY of the criteria listed below are met:

- Prior CABG
- Hematocrit <30 at baseline (ANH Group only). Hematocrit will be assessed by anesthesiologist and surgeon to determine if patient is eligible for control group
- Insufficient (Low) on pump hematocrit of <21%
- Patient is hemodynamically unstable
- Subject requiring an emergency procedure
- Left main coronary artery stenosis with evidence of hemodynamic instability (e.g. hypotension, ST segment elevations on electrocardiogram).
- Aortic valve stenosis (e.g. hypotension, ST segment elevations on electrocardiogram).

3. STUDY METHODS AND PROCEDURES

3.1 Study Procedures

This trial involves serial blood sample collection starting prior to anesthesia administration until 16 to 24 hours post- surgery A total of 60- 80 mLs of blood will be collected for research purposes throughout the study duration based on group designation (ANH recipients versus control). See timing of assessments table for specific laboratory tests drawn at each time pointfor each group.

The screening process will consist of the routine pre-operative screening process which is standard of care for cardiopulmonary bypass surgery: imaging, laboratory blood draws and a complete history and physical examination. Those patients who meet all of the inclusion criteria and do not meet any of the exclusion criteria will be offered enrollment.

Baseline shall be defined as the commencement of CABG surgery upon the induction of anesthesia, but before utilization of the coronary artery bypass machine. Baseline laboratory assessments which will entail collection of the markers of coagulation and platelet function at the commencement of surgery upon induction of anesthesia and the insertion of the central venous catheter. During CPB just after the induction of anesthesia, a central venous catheter and other monitoring catheters are inserted into the patient. The insertions of these monitoring catheters are standard of care during CABG surgery. Prior to the start of the blood removal phase of the ANH procedure, blood samples will be obtained for baseline complete blood count, coagulation, inflammation, and hemostatic markers. A calculated amount of blood will then be removed and sequestered at room temperature in the cardiovascular operating room (CVOR) to protect it from the damaging effects of both the CPB machine and the procedure.

Once the CPB machine has been initiated, the subjects on- pump hematocrit (HCT) will be calculated. A safety threshold for blood removal in the ANH process will be calculated according to the following formula by the perfusionist:

$$EBV = fbv \times kg \qquad \text{Eq.1}$$

$$\text{Hct pre-cpb} = \text{Hct on cpb} \times \frac{(EBV + \text{Prime volume})}{EBV} \qquad \text{Eq.2}$$

$$\text{ANH Volume} = \frac{EBV \times (\text{Hct baseline} - \text{Hct pre-cpb})}{\text{Hct baseline}} \qquad \text{Eq.3}$$

- EBV= patient's blood volume in milliliters.
- Fbv= blood volume factor in cc blood per kilogram: usually 65-70ml
- Kg = patient's weight in kilograms
- Hct pre-cpb = percent estimated patient Hct required before CPB to achieve the desired Hct on cpb

- Hct on cpb = desired percent Hct after initiating CPB
- Prime volume = extracorporeal circuit prime volume after retrograde autologous priming (if applicable).
- Hct baseline = patient's percent Hct after hemodilution after the induction of the anesthetic agent
- ANH volume = whole blood volume in cc to be sequestered prior to CPB

This assessment will determine whether subjects undergo ANH or do not have sufficient hematocrit to undergo ANH. Once determination of adequate on- pump hematocrit has been confirmed blood samples from the subject will be collected 30 minutes post-CPB machine initiation, once immediately off of CPB, and post protamine infusion and after ANH reinfusion. Further draws will be obtained at 1 hour post reinfusion, 2-4 hours post reinfusion, and finally at 16-24 hours post reinfusion in the experimental group In the control group blood samples from the subject will be collected 30 minutes post-CPB machine initiation, once immediately off of CPB, and post protamine infusion. Further draws will be obtained at 1 hour post CPB completion, 2-4 hours post CPB completion, and finally at 16-24 hours post CPB completion. While many of these blood draws are standard of care, others are trial-related (i.e. coagulation, inflammation, and hemostasis markers).

The blood tests that are done at 1 and 2 hours post reinfusion are expected to be done in the CVOR or in the Cardiovascular Intensive Care Unit (CVICU) depending on the patient's location. These samples will be obtained through the central venous catheter. The 16-24 hour blood draw will be done in the (CVICU) through the central venous catheter or phlebotomy depending on the time of discontinuation of the central line, and this will conclude blood sampling collection for the trial.

Should the subject become hemodynamically unstable (systolic BP less than 90 mmHg) at any time within either the experimental or the control group assessments will be stopped and standard of care will be continued as appropriate for the subject's condition. Patient will continue to be followed for 30 days or as their condition dictates as per standard of care.

Upon hospital discharge, medical records will be reviewed to record discharge date, assess for adverse events as specified in section 7.1, and to document the total number of allogenic blood transfusions.

Should the subject consent to the use of remaining specimens, plasma samples for possible future analysis will be stored at minus 70- 80 degrees Celsius for up to 2 years.

3.2 Endpoints/Outcomes Measurements

3.2.1 Primary Objective

- The primary endpoint will be assessment of hemostasis at baseline and serially during CABG surgery utilizing the CPB.

3.2.4 Informed Consent

The research staff will meet with the participant to discuss the goals of the study, possible risks of participation, and to answer any related to the procedure or to the study. The research staff will allow adequate time and privacy for decision making and then obtain a signed consent document. The subject will receive a copy of the signed consent form. Patients incapable of providing consent due to a medical situation will not be approached for study participation. No study related procedures may commence without a signed consent form. A separate signature will be required to document a subjects agreement to allow any remaining specimens obtained during the course of participation to be used for ancillary research. Subjects who decline to participate in this optional research will not provide this separate signature and any remaining specimens will be destroyed.

4. STATISTICAL CONSIDERATIONS/DATA ANALYSIS

4.1 Statistical Calculations

Although existing data identifies a positive correlation between ANH and a reduced need for allogenic transfusion, the procedures effect on markers of coagulation and platelet activation in patients undergoing CABG surgery remains untested. As such a reasonable calculation of a sample size based on the accepted error rate is currently not feasible.

4.2 Statistical Analysis

Categorical variables will be expressed as n (%). Continuous variables will be reported as mean±SD, or median (interquartile range), where appropriate. The paired t-test or Wilcoxon signed rank test or will be performed to test differences in lab values before and after treatment. Correlation coefficients will be performed by Pearson's or Spearman's Rho, where appropriate. Predictive cut-point values of platelet function tests to clinical endpoints will be assessed by ROC curve analysis with area under curve (AUC) statistics and 95% confidence intervals presented. Although underpowered given the pilot nature, to address the hypothesis that ANH will be associated with changes in hemostatic markers, reduced chest-tube drainage, reduction in transfusion, etc., we will use separate repeated measures generalized linear mixed model (GLMM) with a random intercept and the appropriate link function (e.g., binary, logit, etc.). Statistical calculations will be carried out using SAS (ver. 9.3, Cary, NC).

4.3 Data Storage

4.3.1 Data Management

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Each subject screened and enrolled will be assigned a subject identification number (SID) and a list of subjects with their corresponding SID will be maintained separately from collected data. Physical CRFs will be stored at the research site in a locked office and electronic subject data will be locked in a password protected file on a secure internet server, accessed only by authorized research staff.

4.3.2 Records Retention

The Investigator will maintain the records of final CRFs (CDROM copies), worksheets, source documents, and all other study-specific documentation in accordance with ICH Guidelines. Essential documents should be retained for at least three (3) years after the investigation is formally discontinued.

5. HUMAN SUBJECTS PROTECTION (Risks, Benefits, and Alternatives)

5.1 Risks

5.1.1 Potential Loss of Privacy

Protected health information (PHI) will be collected during the study. The risk for breach of confidentiality and privacy will be minimized by shielding the subjects unlinking his or her identity from his or her personal health information.

5.1.2 Potential Adverse Events

Aside from additional phlebotomies allowing blood collection as per protocol, no other additional treatments or procedures will be given to subjects for this trial. One research venipuncture maybe necessary to obtain the 16-24 hour blood draw. As such, pain, discomfort, or bruising on the venipuncture site may occur.

5.2 Benefits and Alternatives

There are no direct benefits to the patient. Participation in the study is entirely voluntary. The alternative is not to participate in the trial but to still receive the ANH procedure as part of CABG surgery.

6. SCHEDULE OF ASSESSMENTS

6.1 Laboratory Assessments

- Light transmittance platelet aggregation stimulated by ADP, collagen, TRAP, and Arachidonic acid (at all study time points).
- VerifyNow® platelet function testing, multiple cartridges (Pre CPB, Off CPB, Post-ANH[1], and 16-24hr Post Surgery)
- Platelet Count using Plateletworks system (Pre CPB, Off CPB, Post-ANH[1], and 16- 24hr Post Surgery)
- Hematocrit using GEM 3500® system (Pre CPB, Off CPB, Post-ANH[1], and 16-24hr Post Surgery
- Hemoglobin using HemoCue® $Hb201^{+}$ system (Pre CPB, Off CPB, Post-ANH[1], and 16- 24hr Post Surgery
- Thrombelastography by ROTEM® (Pre CPB, Off CPB, Post-ANH[1], and 16-24 hours post-surgery)
- Calibrated Automated Thrombogram (Pre CPB, just prior to and post- ANH[1])
- Real time evaluation of thrombus formation using by novel T-TAS® plus system (Pre-CPB, just prior to and Post ANH[1])

- Fibrinogen, D-dimer, vWF and CRP (Pre CPB, immediately after ANH[1], 2-4 hours post-surgery, and 16-24 hours post- surgery)

[1] *Post surgical assessments will be collected from both the experimental group and the control group. Post CPB assessments will be collected from the control group at the same intervals (1 hour, 2-4 hours, and 16-24 hours) as the experimental group with the exception of the post ANH and post protamine draw.*

6.2 Health and Concomitant Medication Review

Changes in participant's medical condition (any new diagnosis, etc.) and concomitant medications will be continuously updated via medical records review. Serious adverse events, and clinical endpoints and adverse events specified in section 7.1 will also be continuously assessed during hospital stay.

6.3 Physical Examination

The physical examination is completed as standard of care for preoperative evaluation. Data from the physical exam will be used to evaluate the general status of the subject and to further elucidate patient symptoms, risk factors, or concerns that may increase the subject's risk for adverse events.

6.4 Safety Assessments

Safety assessments will consist of the monitoring and recording of serious adverse events. The occurrence of adverse events will be ascertained via clinical assessment of bleeding. For the purpose of this study, thromboembolic, bleeding events and serious adverse events (SAEs) directly related to cardiac surgery utilizing the CPB machine, and SAEs directly related to study procedures (needle stick) will be collected and reported. Additional assessments required to ensure the safety of subjects will be administered as deemed necessary by the investigator on a case by case basis.

6.5 Schedule of Procedures

The study is comprised of 3 periods: Preoperative and Screening, During Surgery, and Postoperative. The Preoperative and Screening period is comprised of: baseline assessments and assessment of coagulation markers. The During Surgery period is comprised of a baseline before ANH removal, 30 minutes post-CBP start, off CPB, post-protamine infusion, and post-ANH reinfusion timepoints. The Postoperative period is comprised of: 1 hour, 2-4 hours, and 16-24 hours post ANH reinfusion timepoints and routine standard of care evaluation for CABG surgery with longitudinal follow up in STS database. Medical records will be reviewed upon hospital discharge to assess for adverse events and documentation of discharge date. See table below for timing of assessments.

Study Assessment\/Procedure	Preoperative	During Surgery					Postoperative			30 day post-surgery follow up[5]
	Screening	Baseline before ANH removal	30 minutes post CBP start	Immediately Off CPB	Post protamine infusion before ANH	Immediately Post ANH Reinfusion[4]	1 hour post ANH (+15 minutes)	2-4 hours post ANH	16-24 hours post ANH	
Assessments										
Explain Study/ Informed Consent	X									
Review of Inclusion/Exclusion Criteria	X									
Medical History and Demographics	X									X
Physical Examination	X[1]									X
Vital Signs Assessment	X[1]									X

Previous and Concomitant Medications	X									X
Adverse Events Assessment (Bleeding and Non-Bleeding)	→	→	→	→	→	→	→	→	→	X
Serious Adverse Events Assessment (Bleeding and Non-Bleeding)	→	→	→	→	→	→	→	→	→	X
Clinical Efficacy Endpoint Assessment (Bleeding, Transfusion)	→	→	→	→	→	→	→	→	→	X
Standard of Care Local Laboratory Tests										
Hematology[2]	X[1]	X						X	X	
PT/INR, aPTT[2]	X[1]	X						X	X	
Biomarker Testing										
Fibrinogen	X[1]	X				X		X	X	
D-Dimer	X[1]	X				X		X	X	
Von Willebrand Factor	X[1]	X				X		X	X	
C Reactive Protein	X[1]	X				X		X	X	
Platelet Function Testing										
Light transmittance aggregation		X	X[3]	X	X	X	X	X	X	
VerifyNow		X		X		X			X	
Thrombelastography by ROTEM		X		X		X			X	
Platelet count by Plateletworks		X		X		X			X	
Hemoglobin by Hemocue		X		X		X			X	
Hematocrit by GEM 3500		X		X		X			X	
Calibrated Automated Thrombogram		X			X	X				
Real time evaluation of thrombus formation T-TAS		X			X	X				

[1] Data from the preoperative physical exam completed as per standard of care will be collected
[2] Standard of care laboratory measurements
[3] Laboratory collections only from autologous blood collected[4] Post ANH assessments will be collected from the experimental group. In the control group the 'immediately post ANH reinfusion assessment will not be done.
[5] Routine post discharge standard of care office evaluation. Longitudinal evaluation follow- up in STS database

6.5.1 Timing of Assessments

6.5.1.1 Preoperative/Screening

Subjects will be pre-screened to ensure that the subject is eligible for the study. All study inclusion criteria must be met and subject must not meet any exclusion criteria. During the screening visit, the following will be completed and documented in the Case Report Forms (CRF):

- Obtain written informed consent
- Complete inclusion/exclusion criteria
- Obtain demographic information (i.e. date of birth, gender, and race)
- Record medical history
- Record concomitant medications
- Obtain data from pre-operative physical exam, vital signs, height and weight completed as standard of care
- Obtain pre-operative hematology and PT/INR, aPTT results (standard of care laboratory measurements) (See table)
- Complete blood count, Fibrinogen, D-Dimer, von Willebrand factor, and C reactive protein may be collected at screening as long as CABG surgery is less than seven days from the collection date. These assessments will be collected on the day of surgery during the 'Baseline' assessment if screening exceeds the surgery by more than seven days.

6.5.1.2 **Perioperative Period**:

6.5.1.3 **Baseline**

Study will begin when the baseline study hematocrit assessment blood sample drawn in the CVOR in the experimental group and post CPB in the control group. Baseline labs are assed in the experimental ANH recipient group before ANH removal, 30 minutes post-CBP start, off CPB, post-protamine infusion, and post-ANH reinfusion timepoints. Baseline labs are assed in the control non- ANH group before ANH removal, 30 minutes post-CBP start, and off CPB timepoints. During this period, the following procedures will be completed:

- Laboratory measurements collection of markers of coagulation and platelet function. (See Schedule of Assessments for specific laboratory assays needed for each timepoint)

6.5.1.4 **Postoperative Period**:

Blood drawn immediately post completion of ANH reinfusion will define the time point that all post-operative blood draws in the experimental group will be based on, while off CPB will define the time point that all post-operative blood draws in the control group will be based on. Time points for the postoperative period include 1 hour (+ 15 minutes), 2-4 hours, and 16-24 hours post-surgery. The following study procedures will be completed:

- Laboratory measurements collection (see table of assessments for specific laboratory assays needed for each timepoint)
- Record total CT drainage amount in 24 hours post placement
- Record total number of whole blood or packed red cells transfused, if applicable.

6.5.1.5 **Hospital discharge and Follow up:**

Patient will undergo routine post-operative discharge evaluation that is standard of care for all CABG surgeries, and longitudinal follow up in STS database. Patient post-operative evaluation will include all standard of care assessments as well as occurrence of adverse events, serious adverse events and clinical efficacy assessments related to bleeding and total number of blood products infused.

7. ADVERSE EVENTS

7.1 AE Recording

Adverse events will be collected postoperatively. For the purpose of this study, only the following adverse events will be collected and reported:

- SAE's related to study procedures (i.e. SAE's related to phlebotomy procedure)

7.2 Adverse Events Treatment

All AEs should be treated appropriately and managed according to standard of care. The action taken to treat the AE should be recorded on the AE CRF. Assessment should be made at each protocol time point of any changes in severity, relationship to the study procedures (if applicable), and the interventions required to treat it, and the outcomes of those interventions.

7.3 Adverse Event Documentation

The principal investigator has the primary responsibility for SAE identification, documentation, grading, and assignment of attribution to the study intervention. SAEs must be recorded in the AE CRF with the following information:

- AE term
- The intensity grade (CTCAE v 4.03 grading)
- The relationship to study procedure
- Attribution
- Duration
- Occurrence (known risks for study procedure-expected, unexpected)
- Other contributing causes
- Actions in response to event
- Outcome
- Criteria for SAE

7.4 AE Grading Scale

The descriptions and grading scales found in NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 will be used for AE reporting. Each AE term is associated with a 5-point severity scale.

7.5 Protocol Deviations and Violations

The Principal Investigator will not deviate from the protocol without obtaining approval from the IRB or Ethics Committee and the sponsor. Protocol deviations that occur will be reported to the Inova IRB at the time of continuing review. Protocol violations that affect the subject's rights and safety, and/or affects study integrity will be reported to the Inova IRB within 10 working days of event knowledge.

8. STUDY COST

The subject or their insurance company will not be billed for this study. All study related tests that is not considered as standard of care will be paid for by the research site.

9. FUNDING

This is an investigator-initiated study sponsored by Inova Heart and Vascular Institute and Instrumentation Laboratories, Inc. There is no investigational product involved. Treatment received by the subject will be as according to main provider's discretion as according to standard of care.

10. CONFLICTS OF INTEREST

The investigators declare no conflicts of interest linked to this study.

11. FACILITIES AND EQUIPMENT

The research site is equipped with its own laboratory equipment, which includes state of the art technologies for platelet assays, centrifuges, refrigerators, and freezers for study specimen processing and storage.

12. OUTSIDE CONSULTANTS/COLLABORATORS
There are no outside consultants/collaborators participating.

13. CONTRACTURAL AGREEMENTS
There are no outside consultants/collaborators participating.

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