A Randomized Controlled Investigation of the Effects of 6% Hydroxyethylstarch 130/0.4 (Voluven) on Renal Function in Patients having Aortic Valve Replacement with or without Coronary Artery Bypass Grafting

Short Title: U**S**e of 6% **H**ydroxyethylstarch (130/0.4) in C**ar**diac Surgical **P**atients **(SHARP)**

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U**S**e of 6% **H**ydroxyethylstarch (130/0.4) in C**ar**diac Surgical **P**atients **(SHARP)**

Patients who have cardiac surgery with cardiopulmonary bypass (CPB) require significant plasma volume expansion in the perioperative period in response to surgical blood loss and for hemodynamic optimization. For these indications, colloid volume replacement solutions, including hydroxyethylstarch solutions (HES) and human albumin 5%, are commonly administered in the perioperative period. Colloid volume replacement solutions are advantageous because they provide more effective intravascular volume replacement than crystalloid solutions; thus patients who receive HES solutions may require less intravascular volume replacement compared with patients who receive crystalloid solutions. Certainly, efficient use of colloid volume replacement therapy, such as HES, rather than excessive crystalloid solutions, may decrease postoperative fluid overload and associated complications including pulmonary edema and pleural effusions. HES solutions may especially benefit cardiac surgical patients, since fluid overload, pulmonary edema, and pleural effusions occur frequently in the postoperative period and are a frequent cause of prolonged hospital stay and hospital readmissions.

The safety of HES solutions, specifically 6% HES 130/0.4, has recently been questioned. Recent large randomized controlled trials reported increased use of renal replacement therapy (RRT)[1,2] and mortality[2] with HES administration. As a result of these and other recent publications, the FDA has issued a *Boxed Warning* which states *"Do not use HES products, including Voluven®, in critically ill adult patients, including patients with sepsis*". However, these investigations examined septic and high-risk critically ill patients and specifically excluded cardiac surgical patients. Thus, whether these results apply to the cardiac surgical population is unclear. Further, Perner et al.[2] examined HES 130/0.42 (Tetraspan), not HES 130/0.4 (Voluven), and the fact that important pharmacokinetic differences exist between these two products[3] prevents the extrapolation of the clinical effects of one HES product to another. In addition, another interpretation of recent results[1] is that HES 130/0.4 may have acted as a more potent or lasting intravascular volume expander than normal saline which resulted in a higher use of RRT because of over-resuscitation in patients who received HES 130/0.4. Importantly, these reports of increased risk associated with HES solutions conflict with a recent meta-analysis of 59 randomized controlled trials of adult and pediatric surgical patients who received modern HES solutions (including HES 130/0.4) and found no evidence for renal injury.[4] The absence of kidney injury in elective surgical patients[4] suggests that different clinical conditions may affect safety profiles and that HES may be safe when administered to elective surgical patients who require volume replacement for acute hemorrhagic hypovolemia. Further, a recent investigation in pediatric patients undergoing elective surgery for congenital heart disease found no increased risk of kidney injury in patients receiving HES 130/0.4.[5] Thus the impact of HES 130/0.4 (Voluven) on renal function in adult cardiac surgical patients is unclear. An investigation comparing the safety of HES 130/0.4 (Voluven) on renal function with a routine plasma volume expander, human albumin 5%, in cardiac surgical patients has not been performed in adults.

Coagulopathy following cardiac surgery may significantly impact postoperative outcomes by increasing blood loss, blood product transfusion, and risk of chest reexploration. Cardiac surgical patients are at increased risk for coagulation abnormalities and platelet dysfunction as a result of hemodilution and destruction by cardiopulmonary bypass. Early generation HES solutions have been associated with coagulation abnormalities.[6,7] A recent meta-analysis of 18 randomized, controlled trials found increased risk of bleeding, blood product transfusion, and reoperation in patients who received HES solutions during cardiac surgery.[8] Following this report, the FDA has added safety information, specifically, the risk of excess bleeding in cardiac surgical

patients, to the Warnings and Precautions section of the package insert of all HES products, irrespective of molecular weight or degree of molar substitution.[a] It is important to note, however, that this report was limited by the fact that head-to-head trials comparing risk of bleeding of newer generation HES solutions, such as HES 130/0.4 with human albumin 5% have not been performed. In fact, this analysis[8] extrapolated results from older generation HES solutions (HES 200/0.5) to the newer HES products, because a direct comparison was not available. Thus an investigation comparing coagulation abnormalities and platelet dysfunction between albumin and the newer HES products, namely HES 130/0.4 (Voluven) is necessary to examine the safety profile in adult cardiac surgical patients.

Cardiac surgical patients are also at risk for a vigorous inflammatory response, which, if severe, may significantly worsen postoperative clinical outcomes.[9] This inflammatory response may also contribute to multiorgan system dysfunction that can manifest as pulmonary dysfunction, coagulopathy, renal insufficiency, myocardial dysfunction, and neurocognitive defects.[10,11] Limited information suggests that HES solutions may provide beneficial antiinflammatory effects.[12] Efforts to protect kidney and pulmonary function and coagulation/platelet abnormalities while inhibiting this harmful inflammatory response may improve outcomes following cardiac surgery.

Our hypothesis is that intraoperative administration of HES 130/0.4 (Voluven) in cardiac surgical patients does not increase risk for postoperative kidney injury. Using a double-blind, prospective, randomized, controlled design, **we propose to test the primary hypotheses that intraoperative administration of HES 130/0.4 (Voluven) does not worsen postoperative kidney function** as measured by **urinary concentrations of neutrophil gelatinase-associated lipocalin (NGAL)** in cardiac surgical patients compared with human albumin 5%. Our secondary hypotheses (safety analyses) are that intraoperative administration of HES 130/0.4 does not adversely affect measures of coagulation and platelet function and secondary measures of renal function. In addition, we will examine the effect of HES 130/0.4 (Voluven) on the inflammatory response and postoperative pulmonary function (exploratory analysis).

The **Specific Aims** of this investigation are:

1) **Primary Aim**. **Safety assessment (kidney).** To assess the safety of HES 130/0.4 (Voluven) versus albumin 5% on renal function measured by urinary concentrations of **neutrophil gelatinase-associated lipocalin (NGAL)**, an important marker of renal function, measured at baseline (following anesthetic induction and prior to surgical incision), within one hour (± 1 hour) of arrival to intensive care unit (ICU), and 24 hours (± 2 hours) following completion of surgery (or within 2 hours prior to ICU discharge if discharged in less than 24 hours) compared with baseline.
2) **Secondary Aim 1: Safety assessment (kidney)**. To assess the safety of HES 130/0.4 (Voluven) on additional measures of renal function including urinary concentrations of interleukin (IL)-18 measured at baseline (following anesthetic induction and prior to surgical incision), within one hour of arrival to ICU (± 1 hour), and 24 hours (± 2 hours) following completion of surgery (or within two hours prior to discharge from the ICU if patient is discharged in less than 24 hours). The severity of kidney injury assessed by RIFLE criteria will also be assessed as a secondary outcome. Clinical exploratory outcomes including requirement of renal replacement therapy (RRT) will also be collected.

[a] Hespan (6% HES 450/0.7 in 0.9% Sodium Chloride Injection) is not recommended for use as a cardiac bypass pump prime, while the patient is on cardiopulmonary bypass, or in the immediate period after the pump has been discontinued because of the risk of increasing coagulation abnormalities and bleeding in patients whose coagulation status is already impaired.

3) **Secondary Aim 2: Safety assessment (coagulation and platelet function)**. To assess the safety of HES 130/0.4 versus albumin 5% as measured by coagulation parameters and platelet function measured at baseline, within one hour of arrival to ICU (± 1 hour), and 24 hours (± 2 hours) following surgery (or within two hours prior to discharge from the ICU if patient is discharged in less than 24 hours). Additional exploratory clinical outcomes including blood loss and transfusion requirements will also be collected.

4) **Tertiary Aim 1: Exploratory analyses (inflammatory response)**. To assess the anti-inflammatory effects of intraoperative administration of HES 130/0.4 in cardiac surgical patients measured by perioperative levels of interleukin - 6 (IL-6), tumor necrosis factor - α (TNF-α), and macrophage migration inhibitory factor (MIF), measured at baseline (following anesthetic induction and prior to surgical incision), within one hour of arrival to ICU (± 1 hour), and 24 hours (± 2 hours) postoperatively (or within two hours prior to discharge from the ICU if patient is discharged in less than 24 hours) compared with patients who received 5% human albumin.

5) **Tertiary Aim 2: Exploratory analysis (pulmonary function).** To examine whether intraoperative administration of HES 130/0.4 compared with 5% albumin affords benefit on postoperative pulmonary dysfunction as measured by ratio of partial arterial oxygen tension (PaO_2) to fraction of inspired oxygen (FiO_2) measured at baseline, within one hour of arrival to ICU (± 1 hour), and 6 hours following ICU arrival (± 1 hour) or within 30 min prior to initiation of Continuous Positive Airway Pressure (CPAP) weaning trial prior to extubation , whichever is earlier. Additional exploratory outcome measures including dynamic lung compliance will be assessed.

BACKGROUND

Patients who have cardiac surgery with cardiopulmonary bypass (CPB) require significant plasma volume expansion in the perioperative period in response to surgical blood loss and hemodynamic perturbations. For these indications, colloid volume replacement solutions, including hydroxyethylstarch solutions (HES) and human albumin 5%, are commonly administered in the perioperative period. Colloid volume replacement solutions are advantageous because they provide more effective intravascular volume replacement than crystalloid solutions; thus patients who receive HES solutions may require less intravascular volume replacement compared with patients who receive crystalloid solutions. Certainly, efficient use of colloid volume replacement therapy, such as HES, rather than excessive crystalloid solutions, may decrease postoperative fluid overload and associated complications including pulmonary edema and pleural effusions. HES solutions may especially benefit cardiac surgical patients, since fluid overload, pulmonary edema, and pleural effusions occur frequently in the postoperative period and are a frequent cause of prolonged hospital stay and hospital readmissions, Currently, colloid volume replacement solutions administered during the perioperative period at the Cleveland Clinic are limited to human albumin 5%. Human albumin 5% is an effective but expensive volume replacement solution. If HES 130/0.4 (Voluven) was proven to be a safe volume replacement therapy without adverse effects on renal function, coagulation, or platelet function, then an alternative safe new and effective volume replacement solution would be available for cardiac surgical patients.

HES 130/0.4 (Voluven) is a third-generation hydroxyethyl starch with a lower molecular weight and molar substitution compared with earlier HES products. The benefit of these newly developed solutions includes more rapid metabolism and clearance which purportedly decreases the adverse effects on renal function and coagulation that were associated with earlier HES products. HES 130/0.4 (Voluven) has been used extensively for volume replacement in surgical patients.

Renal effects

Patients undergoing cardiac surgery are at risk of developing postoperative renal dysfunction. Multiple perioperative risk factors, such as the duration of CPB and low cardiac output, increase risk for postoperative renal insufficiency. Acute cellular inflammation and the perioperative inflammatory response may also play a critical role in the development of post-CPB acute renal insufficiency. Administration of HES solutions with a higher molecular weight and higher molar substitution have been linked with worse renal function. Administration of a hyperoncotic HES solution (10% HES (200/0.5)) worsened renal function in critically ill patients with sepsis and increased risk of death at 90 days.[13] However, newer low molecular weight and molar substitution isotonic HES solutions, including HES 130/0.4 have been purported to have lesser adverse effects on renal function. Markers of postoperative renal function were similar between liver transplant patients who received HES 130/0.4 compared with patients who received 5% human albumin.[14] In contrast, a recent investigation found that critically ill patients with sepsis who received fluid resuscitation with HES 130/0.42 were at an increased risk of death and renal replacement therapy compared with septic patients who received crystalloid solutions.[2] Risk for bleeding was also higher with HES 130/0.42. Interestingly, patients who were not in septic shock at time of randomization did not have increased risk of the primary outcome (death and/or dialysis dependence at 90 days). Importantly, HES 130/0.42 (Tetraspan), not HES 130/0.4 (Voluven) was administered in this trial, which differs in structure and pharmacokinetic properties,[3] than HES 130/0.4. Another recent large randomized controlled trial evaluated the effects of HES 130/0.4

(Voluven) in septic and critically ill patients.[1] This investigation reported no increase in mortality; however there was a small but statistically significant increased use of renal replacement therapy (RRT). As a result of these and other recent publications, the FDA has issued a *Boxed Warning* which states, "*Do not use HES products, including Voluven®, in critically ill adult patients, including patients with sepsis*". However, whether these results apply to the cardiac surgical population is unclear: these investigations examined septic and high-risk critically ill patients and specifically excluded cardiac surgical patients, who the investigators considered "too low-risk". In addition, another interpretation of recent results[1] is that HES 130/0.4 may have acted as a more potent or lasting intravascular volume expander and resulted in over-resuscitation and use of RRT for volume removal. Importantly, these reports of increased risk associated with HES solutions conflict with a recent meta-analysis of 59 randomized controlled trials of adult and pediatric surgical patients who received modern HES solutions (including HES 130/0.4) and found no evidence for renal injury.[4] This report[4] suggests that different clinical conditions could affect safety profiles and that HES appears safe when used in elective surgical patients. Further, an investigation in pediatric patients undergoing elective surgery for congenital heart disease found no increased risk of kidney injury in patients receiving HES 130/0.4.[5] In addition, the safety of HES 130/0.4 (Voluven) compared with human albumin 5% in adult cardiac surgical patients is unclear. Thus, the safety of HES 130/0.4 on renal function and coagulation in cardiac surgical patients requires further investigation.

Platelet effects and coagulation

Coagulopathy following cardiac surgery may significantly impact postoperative outcomes by increasing blood loss, blood product transfusion, and risk of chest reexploration. The etiology of a postoperative coagulopathy is multi-factorial including platelet and clotting factor abnormalities related to hemodilution and destruction by cardiopulmonary bypass, as well as the perioperative inflammatory response. Early generation HES solutions have been associated with coagulation abnormalities[6,7] and increased risk of postoperative bleeding. For these reasons, the use of HES solutions during cardiac surgery has been limited.

Recently, new generation HES solutions, such as HES 130/0.4 with lower molecular weight and molar substitution, have been purported to have minimal adverse effects on coagulation parameters.[15,16] A pooled analysis which compared HES 130/0.4 with earlier generation HES found that HES 130/0.4 produced less abnormalities in coagulation, less blood loss and lower transfusion requirements compared with the earlier generation HES solutions.[17] In addition, newer HES solutions have been found to inhibit the inflammatory response, which may further decrease risk of postoperative coagulopathy. However, although some investigations of the coagulation effects of HES 130/0.4 in cardiac surgical patients found minimal effect on coagulation parameters,[15] conflicting evidence exists.[18] Some reports found that HES 130/0.4 caused global extrinsic and intrinsic pathway coagulation abnormalities in contrast to albumin.[18] In addition, a large randomized controlled trial found that HES 130/0.42 (Tetraspan) was associated with increased incidence of severe bleeding in septic critically ill patients.[2] Further, a recent meta-analysis of 18 randomized, controlled trials found increased risk of bleeding, blood product transfusion, and reoperation in patients who received HES solutions during cardiac surgery.[8] This report was limited, however, by the fact that head-to-head trials comparing risk of bleeding of newer generation HES solutions, such as HES 130/0.4 with human albumin 5% have not been performed. In fact, the analysis extrapolated results from older generation HES solutions (HES 200/0.5) to the newer HES products, because no direct comparison was available. Nevertheless, because of these recent results, the FDA has added safety information, specifically, the risk of excess bleeding in cardiac surgical patients, to the Warnings and Precautions section

of the package insert of all HES products,[b] irrespective of molecular weight or degree of molar substitution. Thus an investigation comparing coagulation abnormalities and bleeding between albumin and the newer HES products, namely HES 130/0.4 (Voluven) are necessary to examine the safety profile in cardiac surgical patients.

Thus, whether HES 130/0.4 (Voluven) impairs clinical and laboratory coagulation parameters compared to albumin in adult cardiac surgical patients is unclear and the clinical impact is unknown. Further investigation of the safety of HES 130/0.4 administration on coagulation parameters is needed.

Anti-inflammatory and pulmonary effects

Cardiac surgery and the use of cardiopulmonary bypass (CPB) incites a vigorous inflammatory response, which, if severe, may significantly impact clinical outcomes.[9] This inflammatory response is mediated by increased secretion of cytokines, including IL-1β, IL-6, IL-8, TNF-α, and MIF. Indeed, a significant inflammatory response may produce multiorgan system dysfunction that can manifest as coagulopathy, respiratory failure, myocardial dysfunction, renal insufficiency, and neurocognitive defects.[10,11] Efforts to inhibit this harmful inflammatory response during cardiac surgery may improve postoperative outcomes.

Recent evidence has found that administration of HES solutions may blunt the perioperative inflammatory response. In an animal model of septic shock, administration of HES resulted in significant anti-inflammatory effects documented by inhibition of TNF-α, IL-1β, and other inflammatory markers. These effects were mediated by inhibition of nuclear factor kappa B (NF-κB) activation.[19] In humans, patients who received perioperative HES 130/0.4 experienced decreased production of matrix metalloproteinase following large bowel surgery.[20] If HES 130/0.4 administration during cardiac surgery could reduce the harmful perioperative inflammatory response, postoperative clinical outcomes may be improved.

The perioperative inflammatory response induces postoperative pulmonary dysfunction by increasing capillary permeability, increasing extravascular lung water, impairing oxygenation and pulmonary compliance. The impact of the inflammatory response on pulmonary function has been correlated with postoperative MIF concentrations, which are inversely related to the postoperative PaO2/FiO2 ratio and directly related to duration of mechanical ventilation and degree of cardiovascular impairment.[21] Inhibition of the perioperative inflammatory response may improve postoperative respiratory function leading to decreased mechanical ventilation requirements and pulmonary morbidity.

HES solutions have been found to have a beneficial effect on pulmonary vascular permeability. In rats, HES (200/0.5) reduced endotoxin-induced increases of lung capillary permeability by inhibiting lung neutrophil accumulation, cytokine-induced neutrophil chemoattractant protein, and NF-κB activation.[22] Other investigations in animals similarly found that use of HES 130/0.4 significantly reduced pulmonary capillary permeability, wet to dry weight ratio, and production of IL-6.[23] In patients with early acute respiratory distress syndrome, HES 200/0.5 significantly improved hemodynamics and cardiac output and attenuated pulmonary vascular permeability.[24] Lung microvascular permeability and neutrophil influx in rats who developed lung injury from high tidal ventilation was attenuated by HES 130/0.4.[25] HES 130/0.4 administration to patients with sepsis was associated with improved measures of oxygenation.[26] Benefits of HES 130/0.4 on pulmonary dysfunction in the postoperative cardiac surgical setting is

[b] Hespan (6% HES 450/0.7 in 0.9% Sodium Chloride Injection) is not recommended for use as a cardiac bypass pump prime, while the patient is on cardiopulmonary bypass, or in the immediate period after the pump has been discontinued because of the risk of increasing coagulation abnormalities and bleeding in patients whose coagulation status is already impaired.

unclear. If perioperative administration of HES solutions attenuated postoperative pulmonary vascular permeability, postoperative pulmonary function and the risk of postoperative pulmonary morbidity may be improved.

Thus, our hypothesis is that intraoperative administration of HES 130/0.4 (Voluven) in cardiac surgical patients does not increase risk for postoperative kidney injury. Using a double-blind, randomized, controlled design, **we propose to test the primary hypotheses that intraoperative administration of HES 130/0.4 (Voluven) does not adversely affect postoperative kidney function** as measured by the primary outcome, **urinary concentrations of neutrophil gelatinase-associated lipocalin (NGAL),** in cardiac surgical patients compared with human albumin 5%. Our secondary hypothesis (safety analysis) is that intraoperative administration of HES 130/0.4 does not adversely affect measures of coagulation and platelet function and secondary measures of renal function. In addition, our investigation will examine the effect of HES 130/0.4 (Voluven) on the inflammatory response and postoperative pulmonary function (exploratory analysis).

The purpose of this investigation is to evaluate the safety of HES 130/0.4 (Voluven) on postoperative renal function, coagulation parameters, and platelet function. The benefits of administration of HES 130/0.4 on the perioperative inflammatory response and pulmonary function will also be examined.

RESEARCH DESIGN and METHODS

Using a double-blind, prospective, randomized, controlled design, the research plan will compare short-term clinical and laboratory outcomes related to an important marker of renal function**, urinary concentrations of NGAL** in cardiac surgical patients who receive HES 130/0.4 (Voluven) for plasma volume expansion during the intraoperative period compared with patients receiving human albumin 5% (primary aim). The safety of HES 130/0.4 on additional markers of renal function, coagulation parameters, and platelet function compared with human albumin 5% will be explored (secondary aim, safety analysis). The benefit of HES 130/0.4 on **inflammatory markers and pulmonary function (tertiary aim, exploratory analysis)** will also be evaluated. One hundred-forty patients will be enrolled (please see sample size analysis).

Patients scheduled for cardiac surgery will be approached by a research nurse or physician in the Cleveland Clinic preoperative cardiac surgical clinic about enrollment into this trial according to the following criteria:

Inclusion criteria

1) Age 40 – 85 years old
2) Scheduled for elective aortic valve replacement with or without coronary artery bypass grafting with or without additional minor surgical procedure.
3) Written, informed consent for participation in this investigation.

Exclusion criteria:

1) Patients with renal failure with oliguria or anuria not related to hypovolemia.
2) Patients receiving dialysis.
3) Patients with preoperative renal insufficiency (Creatinine > 1.6 mg/dL)
4) Anticipated deep hypothermic circulatory arrest
5) Known hypersensitivity or allergy to hydroxyethyl starch or the excipients of hydroxyethyl starch

6) Clinical conditions with volume overload (e.g., patients in pulmonary edema or congestive heart failure)

7) Patients with severe hypernatremia or severe hyperchloremia

8) Patients with intracranial bleeding

9) Pregnant or breast feeding women

10) Critically ill adult patients, including patients with sepsis, due to increased risk of mortality and renal replacement therapy, (e.g. patients who are hospitalized in the intensive care unit prior to surgery)

11) Severe liver disease

12) Pre-existing coagulation or bleeding disorders

13) Any contraindications to proposed interventions.

Randomization

Cardiac surgical patients will be randomized to receive perioperative treatment for acute hypovolemia with HES 130/0.4 (Voluven) during the intraoperative course versus standard therapy (human albumin 5%). Treatment assignments will be generated using a reproducible algorithm in the PLAN procedure in SAS statistical software. Blocks will be of random size from 2 to 6 patients. Randomization will be conducted using an existing password-protected web randomization site used for all clinical trials coordinated by the Department of Outcomes Research and maintained by the Anesthesia Institute statistical team.

Blinding and unblinding

Because of concern of performance bias related to use of hydroxyethyl starch solutions in the cardiac operating room, blinding of the clinician to the type of solution will minimize systematic differences in the care provided to patient groups other than the intervention under investigation. Thus clinicians will be blinded to the randomization regarding which plasma volume expander (study solution) their patients will receive. Because human albumin 5% and HES 130/0.4 (Voluven) are different in color and container, these solutions will be removed from their original containers by the Cleveland Clinic Investigative Pharmacy and transferred into similar flexible containers under sterile conditions. The study solution will be used within 24 hours from time of repackaging or it will be discarded. The Cleveland Clinic Pharmacy is USP 797 compliant with ISO 5 through ISO 7 airflow to provide sterile conditions for IV compounding, the highest practical standard common in hospital pharmacies. The study solution will be labelled with a code that is linked to a record of the patient's randomization status, which will be kept by the Outcomes Research statisticians. For administration of study solution in the cardiac operating room, the study solution will be covered by a shroud and aluminum foil will be wrapped around the intravenous tubing to conceal its contents. Study personnel who are not involved in the care of the patients will insert intravenous infusion sets into the study solution containers in preparation for administration by the anesthesia team in order to maintain anonymity of the study solution. The entire fluid chamber will remain hidden from the anesthesia team by the shroud. The lower area of the fluid chamber of the intravenous infusion set will be covered by an opaque adhesive tape to further obscure the identity of the solution.

The code may be broken in case of medical emergency or if knowing the treatment allocation would influence the treatment of the subject or if demanded by the subject. The code may be broken by contacting the Outcomes Research statisticians. Relevant personnel (i.e. the principal investigator, the Executive Committee) will be alerted to the request to unblind a patient.

If a code is broken, the person requesting this information and the reason for breaking the code will be recorded.

Blinding Questionnaire

To assess the adequacy of blinding, the anesthesia provider (either fellow, resident or certified nurse anesthetist) will be asked to respond to a questionnaire regarding which study solution they think was administered to a given patient. The anesthesia staff will not be assessed for blinding. Assessment will be done following admission of the patient to the intensive care unit following surgery [when no more study intervention will be done for that patient]. Options for the assessment include 1) strongly believe voluven, 2) believe voluven 3) believe albumin, 4) strongly believe albumin, or 5) do not know. Participants will be encouraged NOT to choose option 5) unless they honestly have no idea.
Postoperative primary and secondary outcome variables (urine and blood laboratory measures) will be collected by an unblinded data collection team; however, laboratory analysis by the Cleveland Clinic Research Laboratory of these outcomes will be blinded to randomization group. Data collection of later postoperative events (>2 hours post-operatively) will be blinded to the randomization status.

Outcomes and Aims

Primary Outcome

The **primary outcome measure (primary aim, safety assessment)** to compare the safety of HES 130/0.4 (Voluven) with human albumin 5% on perioperative renal function will include:

1) Urinary concentrations of **neutrophil gelatinase-associated lipocalin (NGAL)**, a marker of renal function measured at baseline (following anesthetic induction and before surgical incision), within one hour of arrival to ICU (± 1 hour), and at 24 hours (± 2 hours) following completion of surgery (or within two hours prior to discharge from the ICU if patient is discharged in less than 24 hours).

Secondary Outcomes

Secondary outcome measures 1 (secondary aim #1, safety assessment, kidney function)

To compare the safety of HES 130/0.4 (Voluven) with human albumin 5% on perioperative renal function will include:

1) **Urinary concentrations of IL-18** measured at baseline, (following anesthetic induction and prior to surgical incision), within one hour of arrival to ICU (± 1 hour), and 24 hours (± 2 hours) following completion of surgery (or within two hours prior to ICU discharge, if the patient is discharged in less than 24 hours).
2) Assessment of **postoperative kidney dysfunction using the RIFLE diagnostic criteria**. Patients will be assessed for risk for kidney dysfunction (RIFLE-R), for injury to the kidney (RIFLE-I), failure of kidney function (RIFLE-F), loss of kidney function (RIFLE-L), and end-stage kidney function (RIFLE-E) based on increases in serum creatinine concentrations. Serum creatinine from baseline and the first seven postoperative days will be collected, if available. (Serum creatinine data to be collected from the patient's medical record, Cardiothoracic Anesthesia Registry, or Cardiovascular Information Registry. See Appendix 1 for the definitions of the RIFLE classifications.) RIFLE category based on urine output collected from ICU nursing records will also be assessed (if data is available).

Descriptive variable measures (kidney function) which will be reported by group

1) Requirement and duration of renal replacement therapy during hospitalization will be recorded.

2) We may store blood and urine samples that can be analyzed at a later date for further analysis of kidney function.

Secondary outcome measures 2 (secondary aim #2, safety assessment, coagulation and platelet function)

To compare the safety of administration of HES 130/0.4 (Voluven) with human albumin 5% in cardiac surgical patients on **coagulation and platelet function** as measured by thrombelastometry and platelet aggregometry compared at baseline, within one hour of arrival to ICU (± 1 hour), and at 24 hours (± 2 hours) following completion of surgery (or within 2 hours prior to ICU discharge, if the patient is discharged in less than 24 hours), if available

Secondary outcome measures of the safety analysis examining coagulation and platelet function collected at baseline, within one hour of arrival to ICU (± 1 hour), and at 24 hours (± 2 hours) following completion of surgery (or within 2 hours prior to ICU discharge, if the patient is discharged in less than 24 hours) may include:

1) Coagulation parameters, including serum **prothrombin time (PT),** and **activated partial thromboplastin time (aPTT)**. The **international normalized ratio (INR)** is an clinically important measure, but it is based on the PT; thus it will be reported descriptively, but not included as an outcome.
2) Additional coagulation measures from thromoelastography, including the **reaction time (R value), clot formation time (K value), angle (α), maximum amplitude (MA), clot lysis at 30 min (LY30).** We will collect data on **coagulation index (CI))** and compare between groups, because it is a clinically important measurement. However, the CI is based on the components of thromboelastography (a summary variable); thus it will not be included as an outcome measure.
3) Assessment of **platelet function**, including platelet count, **ADP-, collagen- and arachidonic acid platelet aggregation**

Descriptive variables of transfusion, coagulation and platelet function)

1) Amount of cell saver administration, packed red blood cell administration, fresh frozen plasma, and platelets transfused, chest tube output during surgery and in the first 24 hours following surgery (or until ICU discharge, if the patient is discharged in less than 24 hours).
2) We will store blood samples for later analysis for coagulation and platelet function, possibly including measures of Factor VIII, von Willebrand's factor, thrombin time, fibrinogen, D-dimer.

<u>Tertiary Outcome Measures</u>

Tertiary aim #1 (exploratory analysis, inflammatory response)

To compare whether intraoperative administration of HES 130/0.4 (Voluven) versus human albumin 5% affords beneficial anti-inflammatory effects as measured by an important markers of the perioperative inflammatory response.

The **<u>tertiary outcomes</u> will include** serum concentrations of **IL-6, TNF- α and MIF**, important **markers of the inflammatory response**, to be measured at baseline, within one hour of arrival to ICU (± 1 hour), and 24 hours (± 2 hours) following completion of surgery (or within two hours prior to ICU discharge, if the patient is discharged in less than 24 hours).

Tertiary aim #2 (exploratory analysis, pulmonary function)

To compare whether intraoperative administration of HES 130/0.4 (Voluven) versus human albumin 5% provides beneficial effects on **postoperative pulmonary function** as measured by the **ratio of partial arterial oxygen tension (PaO2) to fraction of inspired oxygen (FiO2)**

($p_aO2:F_iO2$ ratio) measured at baseline (following anesthetic induction), within one hour of arrival to ICU (± 1 hour), and within 30 min prior to initiation of Continuous Positive Airway Pressure (CPAP) weaning trial prior to extubation or 6 hours (±1 hour) following completion of surgery, whichever is earlier, after end of surgery compared to patients who receive perioperative 5% human albumin.

Tertiary outcome measures of pulmonary function

1. p_aO2: F_iO2 ratio

Exploratory outcomes (pulmonary function)

2. Dynamic lung compliance
3. Gas exchange capacity, including oxygenation index and ventilation index

Dynamic lung compliance: Measurements will be performed using pressure-control mode with a driving pressure titrated to achieve a tidal volume (V_T of 6 mL/kg lean body weight), fixed positive end-expiratory pressure (PEEP) of 8 cmH_20, respiratory rate, fraction of inspired oxygen FIO_2, and inspiratory time. Dynamic lung compliance will be calculated from the following formula:

Dynamic compliance = V_T/(PIP – PEEP),

where PIP = peak inspiratory pressure.

Oxygenation index (OI): OI = MAP × FIO_2 /PaO_2

where MAP = mean airway pressure, FIO_2 = fraction of inspired oxygen.

Ventilation index (VI): VI = RR × (PIP – PEEP) × $PaCO_2$/1,000

where RR = the respiratory rate.

Additional descriptive measures will be collected at baseline and throughout the ICU course:

1) Peak intraoperative and postoperative serum lactic acid
2) Peak intraoperative and postoperative serum base excess
3) Weight gain

Descriptive variables (which will be reported by group) that will be collected during hospitalization or at time of hospital discharge (collected from the patient's medical record, Cardiothoracic Anesthesia Registry, Cardiovascular Information Registry), medical records from outside institutions (with patient consent) and/or the Social Security Death Index. These will be reported and compared by group for descriptive purposes.

1) Dose of cardiovascular inotropic and vasopressor infusions administered at end of surgery and during ICU stay, such as epinephrine, norepinephrine, milrinone.
2) New requirement for mechanical circulatory support following surgery
3) Requirement for chest re-exploration
4) Incidence of postoperative pulmonary effusions (diagnosed by chest X-ray or ultrasound)
5) Postoperative requirement for thoracentesis for treatment of pleural effusion

6) Occurrence of new-onset atrial fibrillation requiring medical therapy or cardioversion

7) Duration of surgery and anesthesia

8) Duration of mechanical ventilation (hrs)

9) Duration of stay in intensive care unit (days)

10) Duration of hospital stay (days)

11) Postoperative in-hospital mortality

12) In-hospital morbidities, including

    a. Cardiac morbidity (postoperative myocardial infarction or low cardiac index with a requirement for mechanical circulatory support),

    b. Neurologic morbidity (new postoperative focal or global neurologic deficit confirmed by clinical findings and/or computed tomographic scan),

    c. Renal morbidity (new postoperative requirement for RRT), including reason for the requirement of RRT, and duration of RRT

    d. Infection morbidity (culture-proven pneumonia, mediastinitis, wound infection or septicemia)

    e. Overall morbidity (incidence of one or more of the above morbidities, including death, since early death precludes observation of morbidity)

13) Fitness for hospital discharge (using modified criteria published by Walji et al.[27])

14) Hospital readmission for fluid overload (including pleural effusion) within 30 ± 5 days following surgery

15) Hospital readmission for atrial fibrillation within 30 ± 5 days following surgery

16) Hospital readmission for any cause within 30 ± 5 days following surgery

17) All-cause mortality at 30 ± 5, 90 ± 15 days and one year (±1 month) following surgery from follow-up phone calls, the patient's primary care physician, or the Social Security Death Index.

18) Kidney function measured by serum creatinine and requirement for RRT and duration of RRT at 90 (± 15) days following surgery collected from medical records at the Cleveland Clinic or outside hospitals if available. Kidney function may be classified into RIFLE categories if adequate long-term data is available (RIFLE-L and RIFLE-E require follow-up data for 4 weeks to 3 months). The reason for RRT will be documented.

19) Renal function measured by serum creatinine and need for RRT between 6 months (±1 month) and 1 year (±1 month) following surgery collected from medical records at the Cleveland Clinic or outside hospitals/physician records if available. Kidney function may be classified into RIFLE categories if adequate long-term data is available. The reason for RRT will be documented.

Experimental protocol

Patients will be randomized into either the HES 130/0.4 (HES) or standard care group (human albumin 5%) prior to induction of anesthesia (see Randomization above). The study drug (HES 130/0.4 (Voluven) or human albumin 5%) will be delivered to the operating room in similar

packaging and covered with a shroud to conceal the appearance of the solution (see Blinding and Unblinding above).

Intraoperative Fluid Management

On arrival to the operating room, a large-bore intravenous catheter will be inserted. Routine administration of crystalloid fluids will occur throughout the intraoperative period as a continuous infusion to provide carrier solutions for intravenous drugs, inotropic and vasopressor agents, and continuous flow to maintain patency of intravascular catheters. This routine crystalloid administration will consist of approximately one liter of fluid prior to initiation of cardiopulmonary bypass and one to two liters of crystalloid following cardiopulmonary bypass.

Following separation from cardiopulmonary bypass, the patient will be continuously monitored for acute hypovolemia requiring plasma volume replacement. Plasma volume replacement using 250 mL increments of the blinded study drug (HES 130/0.4 or 5% human albumin in similar containers and covered by a shroud) will be administered when indicated by any of the following clinical conditions (Figure 1): 1) the cardiac output/cardiac index experiences a 20% decrease from baseline; 2) the heart rate increases to 20% above baseline; 3) mean or systolic blood pressure decreases 20% from baseline; 4) a 20% increase in vasopressor requirement occurs; 5) a 20% decrease in cardiac filling pressures (central venous pressure, pulmonary artery diastolic pressures). When these conditions are met, the patient will receive 250 – 500 mL of study solution (HES 130/0.4 or 5% human albumin) as a fluid bolus. These parameters will be assessed on a continuous basis during the intraoperative period and the patient will receive the study solution each time these conditions are met. We anticipate that an average patient will receive approximately 750 – 1000 mL study solution during a routine cardiac surgical procedure.

Fluid challenges may be repeated until no further increase in these parameters occurs, suggesting that fluid resuscitation has been successful and euvolemia has been achieved. Also, patients will receive fluid equivalent to that judged to be lost from surgical hemorrhage. The maximum dose of HES 130/0.4 will be 35 mL/kg/day. If further plasma volume expansion is needed, lactated Ringer's solution will be used for additional fluid boluses as required.

Transfusion of packed red blood cells and blood components

Minimum hematocrit (HCT) will be approximately 23% following cardiopulmonary bypass and 21% on cardiopulmonary bypass. If the HCT decreases below these values, packed red blood cell transfusions may be administered. Erythrocyte transfusions may be administered for hypovolemia in the setting of a low hematocrit (hematocrit < 26%) or if considered necessary by the attending anesthesiologist or surgeon. Platelets may be transfused for post-CPB clinical bleeding and/or platelet count < 100 after activated clotting time is normalized. Fresh frozen plasma and/or cryoprecipitate transfusion may occur if coagulopathy or severe clinical bleeding persists following protamine administration without evidence for usual hemostatic response, elevated prothrombin time, or elevated activated partial thromboplastin time. In addition, administration of blood component transfusion may be used in the clinical setting of coagulopathy or nonsurgical bleeding as determined by the attending surgeon and/or anesthesiologist.

Anesthetic and surgical management

Anesthetic and surgical patient care will be performed following usual Cleveland Clinic procedures, which include the use of standard ASA monitors, an arterial catheter, and a central venous or pulmonary artery catheter, and transesophageal echocardiography. Intravenous anesthetic induction may consist of etomidate or propofol with succinylcholine or a non-depolarizing muscle relaxant to facilitate tracheal intubation. Isoflurane, fentanyl, and/or midazolam will be administered for maintenance of anesthesia. Additional non-depolarizing muscle relaxant will be administered in incremental doses as clinically indicated. Patients who

require cardiopulmonary bypass may receive aminocaproic acid infusion beginning at induction of anesthesia and continued until 2 – 6 hours following surgery. Routine procedures will be followed for heparinization, arterial and venous cannulation for CPB, commencement of CBP, and myocardial protection strategies. Standard cardiopulmonary bypass prime at the Cleveland Clinic consists of Plasma-Lyte 600 – 900 cc, heparin 10,000 units, sodium bicarbonate 50 mEq, and mannitol 50 g. After aortic cross-clamp removal, epicardial atrial and/or ventricular pacing wires may be placed and lidocaine, magnesium, and/or amiodarone boluses, and direct cardioversion may be administered for ventricular arrhythmias. As per routine, protamine will be administered as 1 mg/100-IU of heparin dose after complete separation from CPB or until heparin effect is neutralized. Arterial and venous decannulation and chest closure will be performed according to routine procedures.

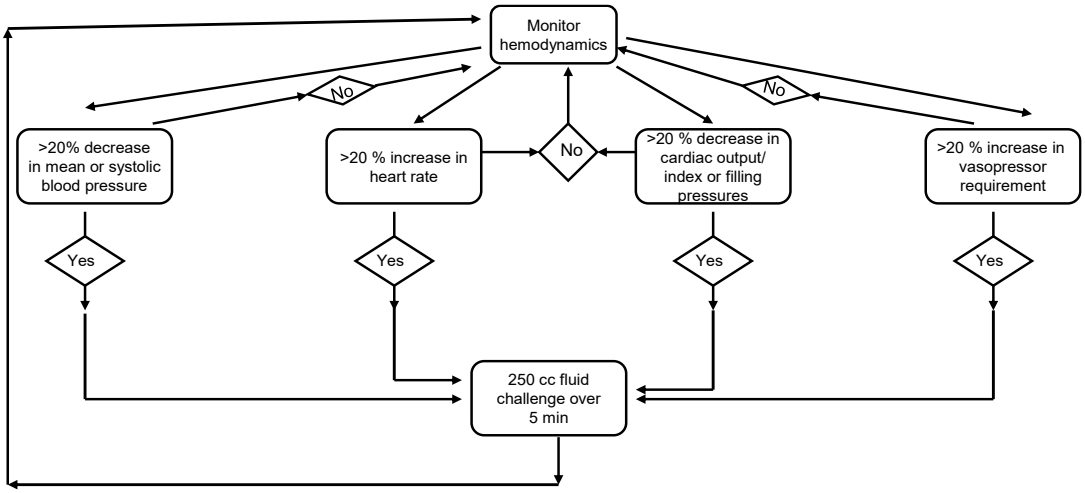
Ventilator management

Protective lung strategy may be used during the intraoperative and postoperative period including tidal volumes of 6 mL/kg (ideal body weight) and positive end-expiratory pressure of 8 mmHg.

Hemodynamic goals and management

Intraoperative hemodynamic targets will be a heart rate between 50 to 100 beats per minutes and a mean arterial pressure >60 and <100 mmHg. The cardiac index (CI) target will be >2.0 L/min/m^2. If cardiac index (CI) is < 2.0 after adequate intravascular volume replacement, then inotropic support with epinephrine or milrinone may be initiated. Usual interventions, including administration of vasodilators (e.g., nitroprusside, nitroglycerine), inotropic agents (e.g., epinephrine, milrinone), vasopressors (e.g., phenylephrine, norepinephrine) for vasoplegia with adequate cardiac index, atrial/ventricular pacing, may be performed if these goals are not met.

Figure 1. Flow chart describing administration of HES 130/0.4 vs. human albumin 5% fluid challenge.



Postoperative Management

On arrival to the intensive care unit, lactated Ringer's solution will be administered postoperatively when the patient demonstrates signs of hypovolemia as described above. Crystalloid fluid may be given as necessary to maintain urine output exceeding 0.5 ml/h per kg ideal body weight. Postoperative pain relief will be maintained in the intensive care unit with intravenous opioids (e.g. fentanyl, morphine). Patients will be weaned from mechanical ventilation and extubated when they fulfill usual criteria that indicate the ability to maintain a patent airway with adequate ventilation and oxygenation. These criteria include an awake, alert, and oriented patient who demonstrates adequate muscle strength with sufficient respiratory mechanics (adequate tidal

volume, respiratory rate). Following tracheal extubation, patients will be given supplemental oxygen via nasal prongs or face mask to maintain oxygen saturation at greater than 92%. Postoperative nausea and vomiting may be treated with intravenous ondansetron 4 mg.

Discharge from the intensive care unit will be determined by the attending intensivist and surgeon, with consideration of the clinical condition of the patient. In all cases, good clinical judgment will predominate and the attending anesthesiologist will modify the protocol as necessary to provide optimal and safe care of the patient.

Definition of time points of laboratory assessment

"Baseline": Baseline urine measurements will occur in the operating room following anesthesia induction and prior to surgical incision and serve as the baseline for comparison of later time points. However, preoperative laboratory blood measurements (e.g., creatinine, coagulation parameters, and other laboratory measurements) that are collected within six weeks of surgery may be used as baseline measurements – with one exception in patients who demonstrate the clinical effect of an anticoagulant agent at the time of laboratory testing. If a patient is treated with anticoagulant medication on day of laboratory testing, coagulation and platelet tests will need to be repeated on day of surgery.

"Following surgery within one hour of arrival to ICU": These laboratory measurements will be measured within one hour (+/- 1 hr) following ICU arrival (after completion of surgery). The Cleveland Clinic standard of care mandates that patients are transferred immediately from the operating room to an intensive care unit upon completion of surgery for reasons of patient safety. The transition from operating room to intensive care unit occurs promptly and smoothly upon completion of surgery. If a delay occurs for any reason, postoperative laboratory measurements (urine NGAL, urine IL-18) will occur within one hour of scheduled (not actual) departure from operating room. This will ensure that postoperative laboratory measurements are collected at the time when peak values are expected, and a delay in transfer of the patient to the ICU will not impact these laboratory results.

"24 hours (± 2 hours) following surgery": These laboratory measurement will be occur at 24 hours (± 2 hours) following completion of surgery defined as time of departure from the operating room following surgery or within two hours prior to removal of central venous/arterial lines and urinary catheter if patient is discharged from the ICU in less than 24 hours.

Justification of laboratory measures of kidney function

NGAL (urine) is an early predictor biomarker of acute kidney injury. Postoperative urine NGAL levels correlated with the severity and duration of AKI, length of stay, requirement for RRT, and mortality in cardiac surgical patients.[28] A postoperative urine NGAL concentration, using a cutoff value of 100 ng/ml, demonstrated excellent sensitivity and specificity. The postoperative urine NGAL levels correlated with severity and duration of acute kidney injury, length of stay, dialysis requirement, and death.

IL-18 (urine) is a biomarker of acute kidney injury that improved risk stratification and identified patients at high risk for progression of acute kidney injury and worse patient outcomes in cardiac surgical patients.[29]

Justification of laboratory measures of the inflammatory response.

IL-6 increases during cardiopulmonary bypass and continues to increase in the first few hours after surgery. Increased IL-6 levels correlate with adverse postoperative outcomes.

TNF-α is an early inflammatory cytokine that initiates the inflammatory response and is pyrogenic. TNF-α facilitates leukocyte–endothelial interaction, and elevation of **TNF-α** and has been

correlated with capillary leak syndrome and induces pulmonary vascular barrier dysfunction, increased lung water content, and impaired oxygenation**.** TNF-α is also associated with myocardial and renal dysfunction.

MIF levels have been related to post-CPB pulmonary and cardiovascular dysfunction, and, increased levels of MIF has been inversely related to the postoperative PaO2/FiO2 ratio and directly related to duration of mechanical ventilation and degree of cardiovascular impairment.

Perioperative Data collection

Baseline variables reflective of the patient's demographic information, medical history, co-morbidities, laboratory values, other descriptive variables, and postoperative complications will be obtained from the patient's medical record, Cardiothoracic Anesthesia Patient Registry of the Department of Cardiothoracic Anesthesia at the Cleveland Clinic or the Cardiovascular Information Registry of the Department of Cardiovascular Surgery. Research use of this Registry is approved by the Institutional Review Board. All perioperative and outcome data in the Cardiothoracic Anesthesia Registry and Cardiovascular Information Registry is collected daily, concurrent with patient care on preprinted forms, by experienced and specifically trained research personnel. Data, which does not conform within a range of expected results, is rejected and reevaluated. This is the routine procedure for data validation for the Registry and ensures that erroneous data is not recorded in the Registry. This data validation procedure is performed in a blinded fashion and will not bias the results. Data will also be collected from the Cardiovascular Information Registry of the Department of Thoracic and Cardiovascular Surgery at the Cleveland Clinic, chart review, and from patient follow-up. The Social Security Death Index will be accessed for mortality data at 30 ± 5 days and one-year ± one month following surgery. A follow-up phone call will be made at 30 ± 5 days to record re-hospitalization for fluid overload, atrial fibrillation, or hospitalization for any other cause and death. A follow-up phone call will be made at 90 ± 15 days and 1 year ± one month to collect information regarding postoperative renal function and to establish one-year survival. We will collect serum creatinine data from all patients who are followed at the Cleveland Clinic who have laboratory measurements of creatinine at no more than one year and no less than 6 months after surgery if available. We will also collect data on the requirement for RRT if available. (Serum creatinine and requirement for RRT day may be used to categorized patients into RIFLE criteria if long-term data is available) For patients who are followed at outside institutions, we will attempt to get the patient's permission to retrieve a serum creatinine measurement from their local health care provider. If the patient has died, the date of death will be recorded to determine whether the patient survived 30 days following surgery.

Criteria to assess fitness for hospital discharge.

Patients will be assessed daily following discharge from ICU on the following criteria to assess fitness for hospital discharge based on discharge criteria published by Walji et al.[27] because these criteria were originally designed to identify cardiac surgical patients in fast-track protocols, they have thus been slightly modified to fit our patient population. These criteria include the following:

1) Stable rhythm for 24 hours
2) Ambulatory
3) Adequate oral intake
4) Stable pulmonary function
5) Afebrile
6) Surgical wounds in satisfactory condition
7) Patient and family comfortable with discharge

Table 1 describes the timing of study procedures and Activities during hospitalization

Activity	Baseline	OR	1 Hour ICU	30 minutes before CPAP trial	6 Hour ICU	12 Hour ICU	24 Hour ICU	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Informed consent	*													
Medical history	*													
Randomization		*												
Blinding		*												
Ventilator settings		*	*											
Arterial blood gas		*	*											
TEG		*	*				*							
LTA		*	*				*							
Urine(NGAl.IL-18)		*	*				*							
Inflammatory markers		*	*				*							
Cell Saver (cc)		*												
Weight	*		*				*	*	*	*	*	*	*	*
Urine output		*			*	*	*	*	*	*	*	*	*	*
BUN/Creatinine	*						*	*	*	*	*	*	*	*
PT/PTT/INR/PLT	*		*				*	*	*	*	*	*	*	*
Medication		*	*		*	*	*							
Questions for 2 Anesthetists		**												
Chest tube bleeding (cc)							*							
Questioner for patient										*	*	*	*	*
RRT								*	*	*	*	*	*	*

Table 2 describes the timing of follow-up data collection after hospital discharge.

	30 days FU	90 days FU	1 year FU
Questioner	*	*	*
Mortality	*	*	*
SAE or AE	*		
Creatinine		*	*
RRT		*	*

Key: TEG = Thromboelastogram; NGAL = Neutrophil gelatinase-associated lipocalin; IL-18 = Interleukin 18; LTA = Light Transmission Aggregometry; BUN = Blood urea nitrogen; PT = Prothrombin time; PTT = Partial thromboplastin time; INR = International normalized ratio; PLT = Platelet count; RRT = Renal replacement therapy; SAE = Serious adverse event; AE = Adverse event

STATISTICAL METHODS

The randomized HES and standard therapy groups will be descriptively compared for balance on potentially confounding baseline variables (including duration of cardiopulmonary bypass, use of anti-fibrinolytic therapy, preoperative anticoagulant medications, including clopidogrel). Any baseline variable for which the standardized difference (i.e., observed difference in means or proportions divided by the pooled standard deviation of the outcome) is greater than 0.25 will be considered imbalanced and adjusted for as a covariate in each of the below analyses. We will not use P-values to determine baseline imbalance since the P-value is quite dependent on the sample size, whereas the standardized difference is more independent of sample size and thus a better assessment of balance. [A standardized difference of 0.25 is conservative (should be 0.39) based on the expected 2.5th and 97.5th percentiles of the sampling distribution, i.e., $1.96 \times \sqrt{2 / \text{n per group}=50} = 0.39$.][30] For all analyses of continuous variables, transformations of the data will be made as appropriate to achieve normality and/or meet other model assumptions. If transformations are not successful, non-parametric analyses analogous to those described below will be employed. For example, we might employ a Wilcoxon rank-sum test to compare the groups on IL-6 at a particular time point if the data are not close to a normal or log-normal distribution.

All primary analyses will be modified intent-to-treat (M-ITT), such that the groups being compared will be all patients who are randomized and who undergo surgery. Analyses of the safety outcomes in Aims 1 and 2 will be noninferiority assessments, and so 1-tailed. All tertiary analyses will be 2-tailed tests for superiority. Conclusions about the effect of HES will be confirmatory for the primary outcome (urinary NGAL) and more exploratory for other outcomes. We will also conduct a "per protocol" analysis for the primary outcome and describe whether these results differ from the modified intent-to-treat results. In the per-protocol analysis we will analyze patients according to the treatment received as oppose to the randomized treatment, and only those patients who complete the entire trial according to the protocol will be included. In case of a discrepancy, the M-ITT results will be primary.

Type I error. The significance level will be 0.025 for the 1-tailed noninferiority tests for the primary outcome and for each set of the secondary outcomes, and 0.05 for each of the 2-tailed superiority tests for tertiary outcomes (i.e., no adjustment for multiple testing for tertiary outcomes).

Our primary hypothesis is that HES 130/0.4 is noninferior to standard care effects on **mean urinary NGAL** measured within one hour of arrival to ICU and 24 following completion of surgery. We a priori define the noninferiority delta for urinary NGAL as a ratio of geometric means (since NGAL data are expected to be lognormal) of 1.15, for an effective delta of 15% of the control group mean. [Note: for this and other outcomes, if the data are non-normal, a log-transformation will be attempted and the treatment effect (if normal on the log-scale) will be summarized as the ratio of geometric means, equivalent to the difference in means of the log-transformed data.] As noted below under "Continuous outcomes measured over time (Primary and Secondary Aims)", we will assess the treatment effect for the primary outcome in the context of a linear mixed model which allows us to simultaneously assess the effect across the collapsed time points if there is no group-time interaction, or else at specific times if there is an interaction detected. We will conduct tests for noninferiority using treatment effect estimates from the linear mixed model and applying 1-tailed t-tests for noninferiority as described in Mascha and Sessler (2011) [Mascha EJ, Sessler DI: Statistical grand rounds: Equivalence and noninferiority testing for regression models and repeated measures designs. Anesth Analg 2011; 112: 678–87], and with t-statistic as follows:

$T_{NI} = \frac{\hat{\beta}_1 + \delta}{SE_{\hat{\beta}_1}}$, where $\hat{\beta}_1$ is the estimated treatment effect with standard error $SE_{\hat{\beta}_1}$ and noninferiority delta of δ.

In Secondary Aim 1 – **kidney function** – we will assess noninferiority of HES to albumin on each of urinary concentration of Il-18 and acute kidney injury as classified by RIFLE criteria, using statistical methods reported below under continuous (urinary concentration of Il-18) and ordinal (RIFLE criteria) outcomes. These will be 1-tailed tests for noninferiority, using a noninferiority delta of 15% of the mean (or a ratio of geometric means of 1.15, if data are found to be log-normally distributed) for urinary concentration of Il-18 and a noninferiority delta of 1.15 for the odds ratio assessing the effect of HES 130/.04 on the ordinal RIFLE outcome. The noninferiority delta of 1.15 corresponds to a 15% higher odds of having a worse RIFLE score with HES 130/.04 versus standard, and is roughly analogous to the 15% difference utilized for the continuous outcomes (urinary NGAL and Il-18). A Bonferroni correction will be made to preserve alpha at 0.025 for this aim.

In Secondary Aim 2, the safety outcomes of **coagulation and platelet function** each comprise a set of several variables. Noninferiority of HES to albumin will be assessed for each outcome at the 0.025 level using a noninferiority delta of 15% of the mean (as explained above). We will claim noninferiority of HES to standard within a set if noninferiority is shown on all of the variables in a set. Therefore, no adjustment for multiple testing within a set will be done (i.e., intersection-union test).

Safety conclusions will be based on descriptive statistics as well as statistical tests. Multivariate analyses considering a vector or set of related outcomes may also be performed.

For tertiary aims all comparisons will be 2-tailed tests for superiority and analyses will follow the methods outlines below based on type of outcome variable. Each test will be conducted at the 0.05 significance level since these are exploratory analyses.

Continuous outcomes measured over time (Primary and secondary aims). For the primary outcome (urinary NGAL) and each of the secondary continuous outcomes which are measured over time (e.g., at baseline, within one hour of arrival to ICU following surgery, and 24 hours following completion of surgery), we will assess the treatment effect of HES 130/0.4 versus standard care using linear mixed effects models (with patient as random effect), adjusting for the baseline value of the outcome and any baseline variables for which balance among the randomized groups was not achieved through randomization. We will adjust for the baseline value of the outcome for efficiency, i.e., to obtain a smaller standard error for the estimate of treatment effect to the extent that the baseline value is correlated with the later time points, regardless of baseline balance. An unstructured within-subject correlation structure will be employed, estimating a distinct correlation parameter for each pair of post-baseline measurement times.

If the treatment group-by-time interaction is significant (P<0.15), the treatment effect for a particular outcome will be assessed at each of the post-baseline time points measured, using a Bonferroni (or other appropriate) correction for multiple comparisons procedure if comparing groups at specific time points. Similarly, correction to the significance criterion will be made in all analyses below when comparing multiple times, as appropriate.

Ordinal outcome. Groups will be compared on the ordinal outcome of the RIFLE criteria (Secondary Aim 1) using proportional odds logistic regression, adjusting for baseline imbalance

as needed. We will assess noninferiority using the approach described above for the primary outcome, where $\hat{\beta}_1$ is the estimated treatment effect in the form of a log-odds ratio.

Time to event outcomes. Randomized groups will be compared on time to event outcomes including duration of mechanical ventilation, duration of stay in the intensive care unit, and duration of hospital stay using Cox proportional hazards regression models adjusting for any baseline imbalances covariables. For these models the outcome will be time to experiencing the event while alive, i.e., time to removing the ventilator, discharge from the ICU and discharge from the hospital. Patients who die before the event occurs will be listed as failures (i.e., no event) and censored at a time just beyond the last observed event for any patient. Ignoring the deaths or censoring at the time of death may introduce bias.

Binary outcomes. Groups will be compared on binary outcomes, including in-hospital cardiac, renal, infection, or neurologic morbidity, and overall major morbidity (including death), using either logistic regression (to adjust for baseline imbalance as needed) or Pearson chi-square or Fisher's exact test, as appropriate. However, the study is not powered for assessing binary outcomes, and these analyses particularly will be interpreted with caution (whether significant or not).

For all analyses, confidence intervals for the estimated treatment effect will be provided, and these, along with the point estimates of treatment effect, will be emphasized in our reporting. SAS statistical software (Carey, NC) and R software version 2.8.1 for Windows (The R Foundation for Statistical Computing, Vienna, Austria) will be used for all statistical analyses.

Missing data. If primary or secondary outcome data are missing we will use multiple imputation of outcome values based on all available data before the time point of interest. As a secondary analysis we will conservatively assign the worst observed value for the given time point across all patients to an HES patient and best observed value to a control patient. If there is a non-trivial amount of missing outcome data we will also conduct sensitivity analyses on various forms of imputation (from none at all to full multiple imputation). However, we will work hard to avoid missing outcome data. If baseline data not included in the analyses are missing we will simply describe the missingness.

Sensitivity analyses. We will conduct a sensitivity analysis for the primary outcome that adjusts for time on CPB, length of surgery and intraoperative hypothermia.

Assessment of adequacy of blinding. We will assess the adequacy of the blinding for blinded study team members involved and delivering the study drug or albumin, i.e., anesthesia staff and either a cardiac anesthesia fellow, anesthesia resident, or certified nurse anesthetist. As explained above, options for the assessment will be 1) strongly believe voluven, 2) believe voluven 3) believe albumin, 4) strongly believe albumin, or 5) do not know. Participants will be encouraged NOT to choose option 5) unless they honestly have no idea.

Besides descriptive assessment of the 5-level questionnaire responses versus actual treatment group, adequacy of the blind will be assessed using 2 blinding indices, the James blinding index31 (JBI) and the Bang blinding index32 (BBI). For each of these we will combined responses 1) and 2) and also levels 3) and 4), to give 3 responses: guess voluven, guess albumin, and don't know.

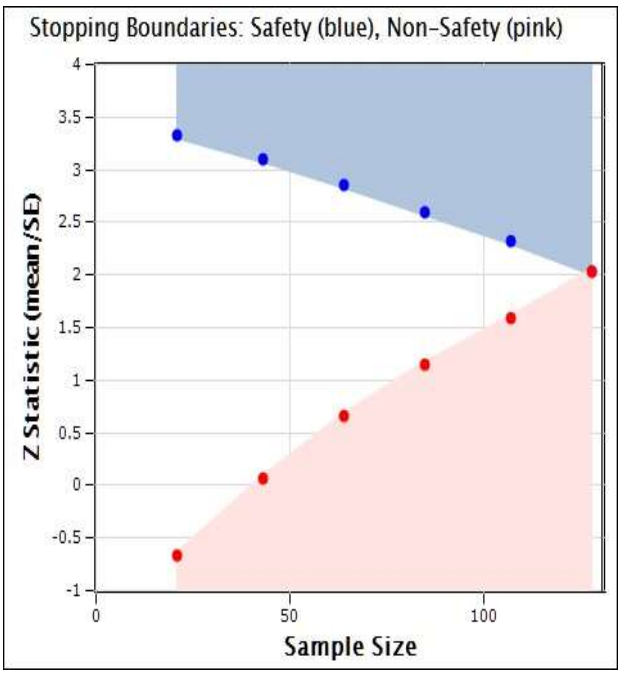
The JBI will be used to assess the blinding of the study overall. It is a modification of kappa designed specifically for the situation of blinding assessment, where correct guesses are least supportive of blinding and therefore assigned a weight of 0, incorrect guesses are moderately supportive of unblinding and therefore assigned a weight of 0.5, and "Don't Know" responses are in fact most supportive of blinding and therefore assigned a weight of 1. The JBI can attain values in the interval [0, 1] with higher values denoting increasing levels of blinding. Therefore, JBI =1 indicates perfect blinding and JBI=0 indicates an unblinded trial.

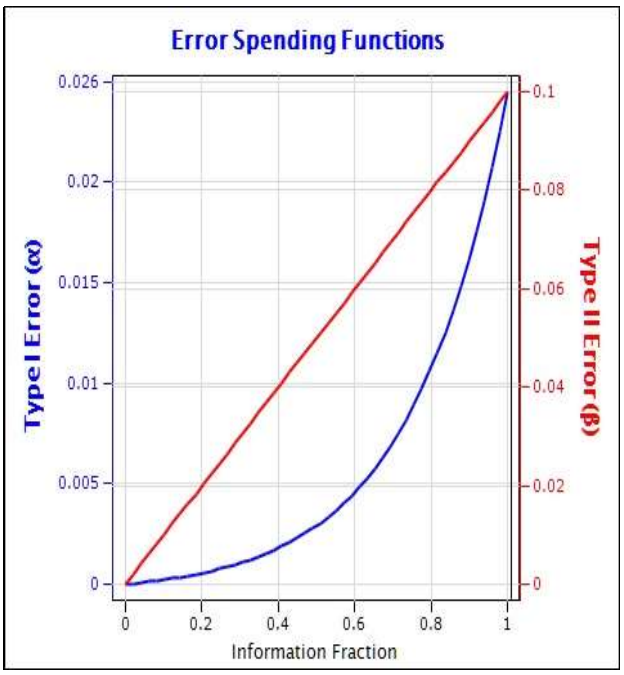
We will also calculate the Bang BI which addresses several critiques of the James BI. In contrast to the JBI, the BBI gives less weight to "Don't Know" responses and more to decisive responses. It is also treatment-arm specific, so it can detect different levels of blinding for patients randomized to Treatment and Control treatment groups. Finally, it is sensitive to "reverse unblinding" in which patients consistently guess the incorrect treatment assignment.

Interim monitoring analyses. We will conduct interim analyses for the parameter urinary NGAL (assessing safety [i.e., noninferiority] and non-safety [i.e., noninferiority not found]) at every 1/6th (16.7%) of the maximum enrollment of 130 patients, or approximately at every 22 or 23 patients, in a group sequential design. We will use the gamma spending function with gamma of −5 for safety (alpha spending; very conservative, like O-Brien-Fleming boundaries) and gamma of 0 for futility, or non-safety (beta spending; fairly aggressive, like Pocock boundaries). We spend the beta faster than the alpha in order to be able to detect any potential harm of the treatment as early as possible in the trial. Table 3 gives the P-value boundaries for efficacy and futility at each interim look (columns 5 and 6). Column 2 gives the total sample size at each look. The last 2 columns give the probability of crossing a boundary when the null hypothesis is true (Under H0) and when the alternative (noninferiority) is true (H1). For example, if Voluven is truly noninferior, the probability of crossing the safety boundary is 3.1% at the first look, 3.1%+12.7% = 16% through the second look, and so on. The below figure displays the boundaries on the z-scale (A) and the error spending functions (B) for this design.

Table 3. Interim Monitoring -- Group Sequential Design Boundaries

Information action	Cumulative Accrual	Alpha Spent	Beta Spent	P-value Boundaries		Boundary Crossing Probabilities	
				Safety H0	Non-safety H1	Under H0 For nonsafety	Under H1 For safety
0.167	22	0.0004	0.017	<0.001	0.746	0.254	0.031
0.333	43	0.001	0.033	0.001	0.471	0.303	0.127
0.500	65	0.003	0.050	0.002	0.256	0.217	0.229
0.667	86	0.006	0.067	0.005	0.125	0.122	0.252
0.833	108	0.013	0.083	0.010	0.056	0.060	0.183
1.000	130	0.025	0.100	0.021	0.021	0.025	0.078





A. Z-statistic Stopping boundaries B. Alpha and beta spending functions

Sample size calculations (Aim 1):

Sample size is based on assessing the noninferiority of HES 130/0.4 to 5% albumin on the primary outcome of urinary NGAL. In patients undergoing cardiac surgery, Koyner et al (2012)[29] observed median [quartile] urinary NGAL values of 28.3 (13.5–82.9) in patients who did not progress to renal failure and 72.1 (11.4–495.6) in patients who progressed to renal failure. Assuming that NGAL values follow a log-normal distribution as in previous studies, we can use the above observed quartiles to estimate the mean, standard deviation and coefficient of variation (CV) for the above groups, yielding CV of 2.3% and 50%, respectively. Assuming for the current study a coefficient of variation between these values, or 25%, we would need 52 patients per group (total of 104) to have 90% power at the 0.025 significance level to be able to claim noninferiority of HES to albumin using a noninferiority delta of a ratio of geometric means of 1.15. These calculations assume no treatment-time interaction and therefore no Bonferroni correction for comparing groups at each of two time points within an outcome. Based on the above calculations, a sample size of 110 would suffice for a study with no interim monitoring. **Adjusting for the interim monitoring at each one-sixth of the total, a maximum sample size of 130 patients are required. Allowing for 5 potential dropouts and 5 pilot patients (which will not to be included in the analyses), we will plan for a potential total of 140 patients.**

STUDY PROCEDURES

Quality Assurance

The major components of quality assurance will be staff training, the standardization of data collection, entry, and processing, and ongoing monitoring to ensure timeliness and accuracy of study data. All study protocols and forms will be compiled into two operations manuals (one for the clinical sites and one for data management) that will be revised as necessary. A clinical reference manual will be created and will include administration and scoring criteria, specific testing instructions and inclusion and exclusion criteria for diagnosis and study ascertainment. This manual will also contain copies of test forms and will be available to all study personnel from a password-protected web site. The current manual for data management will be made easily available to data management personnel and used for continued training. This manual will include a question-by-question written script for interviewing purposes.

Procedures used to assure the integrity of data include: (1) data entry procedures following standard operating procedures (SOPs), and (2) data queries and resolution processes following SOPs as well. Frequent interaction among members of the study Executive Committee (PI, co-investigators, consultants), RAs, and others as necessary, will maintain overall quality assurance. They will meet or have conference calls throughout the data collection period and as necessary thereafter.

Hard-copy forms will be stored in locked cabinets within a secured area. To protect electronic records and files against loss, duplicate files will be maintained on the Division of Anesthesiology servers at Cleveland Clinic. These servers are highly secured because they already contain much patient-related information and are backed up daily to tape which is maintained in a remote location. The system fully meets all applicable HIPAA privacy and security rules. Access to the database and backups are strictly monitored according to need.

Adverse Events

According to Cleveland Clinic IRB Policy – 60, the Institutional Review Board requires Investigators to monitor and report Adverse Events. The Institutional Review Board is responsible to assess changes in risk to ensure safety protections of human subjects

The following definitions are as stated according to Cleveland Clinic IRB Policy.

Definitions

An **Unanticipated Problem Involving Risks to Participants or Others** is any event that (1) is unforeseen, (2) caused harm or placed a person at increased risk of harm, and (3) is related to the research procedures.

An **Adverse Event (AE)** is any untoward or unfavorable medical occurrence, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptoms, or disease. Adverse events encompass both physical and psychological harms.

An **Internal Adverse Event** (AE) is an untoward medical occurrence, which occurs to participants in research conducted by Cleveland Clinic and/or Cleveland Clinic is the IRB of record.

A **Serious Adverse Event (SAE)** is any adverse experience that results in any of the following outcomes:

- death
- a life-threatening experience
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity

- a congenital anomaly/birth defect

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

An **Unexpected Adverse Event** means any AE not previously known or included in the current Investigator's Brochure, consent form or other risk information.

Related/Possibly Related means there must be reasonable evidence to suggest the event was caused by the drug, device or investigational intervention.

Procedures

1. **Internal Serious Adverse Events** (events that occur to participants enrolled in research being conducted by Cleveland Clinic) must be promptly reported to the IRB using the IRB AE Report Form within **10 working days** from discovery/awareness which meet any of the following criteria as assessed by the PI/Co-I:

a) Serious, Unexpected and Related/Possibly Related.

b) AE's determined to be occurring at a significantly higher frequency or severity

than expected.

c) Other Unexpected AE's, regardless of severity, that changes the risk benefit ratio of the study and results in changes to the Research Protocol or Informed Consent process/document.

All Internal SAEs are also reported at **continuing review** using the AE Summary Log.

Patient withdrawal

Patients may be withdrawn from this investigation if they have an adverse reaction directly related to the study drug.

All reasons for each withdrawal will be documented. Patients will have the option to withdraw from treatment only or both treatment and follow-up. Data analysis will follow according to intent-to-treat practice

A patient may choose not to take part in this study or may leave the study at any time. Withdrawing from the study will not result in any penalty nor will it affect the patient's medical care.

EXECUTIVE COMMITTEE

The Executive Committee will function as a primary data and safety advisory group for the trial examining the use of 6% Hydroxyethylstarch (130/0.4) in cardiac surgical patients. The Executive Committee reviews study data, evaluates the treatments for excess adverse experiences according to treatment group, judges whether the overall integrity and conduct of the study remain acceptable and makes recommendations to the **Steering Committee** (see Appendix).

The Executive Committee consists of **three** members (see Appendix). All members have experience and expertise in their field of practice and in the conduct of clinical trials. Two members are experts in the care of cardiac surgical patients, and one member is a statistician.

The Executive Committee will convene to monitor the safety of the trial. This assessment will include a review of all adverse events and safety evaluation. The Executive Committee will make recommendations to the Steering Committee regarding the modification or continuation/discontinuation of the trial. The Steering Committee will review the recommendations of the Executive Committee as outlined in Figure 2. Outcomes Research will maintain the safety

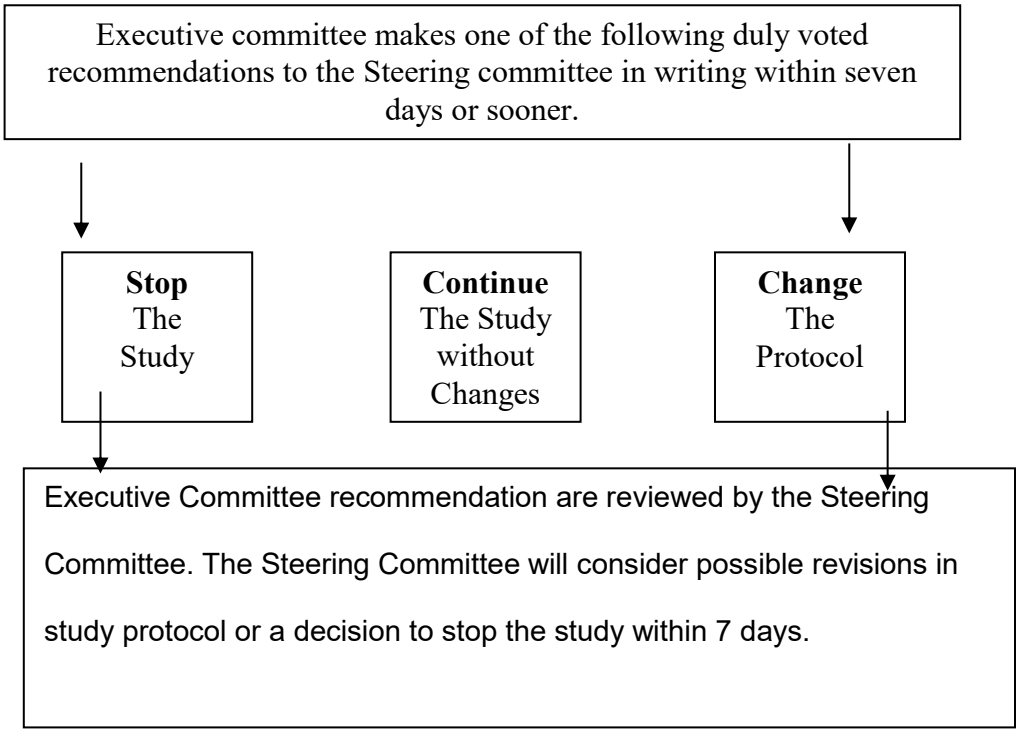
database for the trial. Outcomes Research Statisticians will be responsible for the creation of the Executive Committee safety and efficacy reports and will prepare summary reports of serious adverse events, unanticipated adverse device effects, and study endpoints using blinded treatment groups for the Executive Committee.

The initial review of data by the Executive Committee will take place after at least the first **22** patients have been enrolled and randomized to treatment or control. The Executive Committee will evaluate the data in blinded fashion (e.g. Drug A and Drug B). Subsequent meetings/discussions will be held after approximately every 22 patients are randomized in this investigation. Interim analysis will be planned and pre-programmed in advance ensuring a quick-turnaround. Study recruitment may continue while the interim analysis is being performed. The Executive Committee chairperson and the full committee will determine additional reviews of the data. If necessary, the Executive Committee Chairperson can request more frequent reports.

Figure 2

Actions upon receipt of an

Executive Committee recommendation



Interpretation of safety data is very complex and requires both clinical and statistical experts reviewing the data. A number of considerations for interpretation of these data can be stated and these include:

a) Whether the results could be explained by possible differences in the baseline variables between the groups;

b) Whether outcomes could be biased because of the differences in treatment programs;

c) Whether the results are consistent for other variables which should be associated with the primary outcome variables in question;

d) Whether the results are consistent among various subgroups of patients involved in the study;

e) Whether the risk which is under consideration is outweighed by assessment of the overall benefits of therapy;

f) Whether results could be due to confounding factors and not due to the solution

g) Whether it is likely that the current trends could be reversed if the trial were to be continued unmodified.

All of these considerations require expert evaluation and are the major role of the Executive Committee. The Executive Committee will consider these issues on a regular basis to assure the safety of the patients and to assure the investigators and the medical community that the risks of this study are being evaluated and the patient's safety is being kept foremost in mind. At the point where the Executive Committee believes that evidence of a meaningful difference beyond a reasonable doubt exists between treatment arms such that a specific recommendation related to alteration of the study would be made, a statistician will identify the appropriate treatment groups and the Steering Committee will be notified. The Chairperson will document the discussion with minutes and consolidate the comments of all members. Within three days following each of their meetings, the Executive Chairperson will prepare a written report for the Steering Committee outlining any serious safety concerns and explaining their recommendations. The report will typically include only blinded, pooled-group results, but the committee may release selected unblinded results if considered necessary to the Steering Committee.

Elements of the Executive Committee report may include the following:

1. Patients enrolled

2. Protocol deviations

3. Selected clinical risk/cardiac history factors

4. Selected demographic/baseline factors to include gender, race and age

5. Deaths (patient listing of events to indicate cardiac vs. non-cardiac)

6. Evidence of Kidney injury (urinary concentrations of neutrophil gelatinase-associated lipocalin (NGAL), urinary concentrations of interleukin (IL)-18), serum creatinine, need for renal replacement therapy.

7. Evidence of Coagulopathy or major bleeding (worsening of parameters of coagulation function from thromboelastometry (including the reaction time (R value), clot formation time (K value), **angle (α),** maximum amplitude (MA)**, clot lysis at 30 min (LY30**), and coagulation index (CI) and platelet function and platelet aggregometry including platelet count, ADP-, collagen- and arachidonic acid platelet aggregation), increase in serum coagulation parameters including serum prothrombin time (PT), international normalized ratio (INR), and activated partial thromboplastin time (aPTT),

8. Evidence of liver dysfunction

9. Evidence of anaphylactoid and hypersensitivity reactions

10. Serious Adverse events

Procedures for Communicating Recommendations to the Steering Committee

Executive Committee recommendations that are voted on and passed are transmitted in writing to Andra Duncan, M.D. Chair of the Steering Committee, within three working days (or less if considered urgent) of the meeting/call at which the recommendation was formulated and passed. (Figure 2)

Appendix 1.

Diagnostic criteria for risk of kidney dysfunction

Category	Definition (serum creatinine criteria)	Definition (urine output criteria)
Risk (RIFLE-R)	Increase in serum creatinine x 1.5 from baseline	Less than 0.5 mL/kg/hr for more than 6 hrs
Injury (RIFLE-I)	Increase in serum creatinine x 2 from baseline	Less than 0.5 mL/kg/hr for more than 12 hrs
Failure (RIFLE-F)	Increase in serum creatinine x 3 from baseline	Less than 0.3 mL/kg/hr for 24 hrs or anuria for 12 hrs
Loss (RIFLE-L)	Complete loss of kidney function > 4 weeks	
End stage (RIFLE-E)	End-stage kidney failure > 3 months	

Appendix 2.

Steering Committee members:

Andra Duncan, M.D., Chair, Steering Committee
Department of Cardiothoracic Anesthesia
Cleveland Clinic

Andrea Kurz, M.D.
Vice-Chair, Department of Outcomes Research
Cleveland Clinic

Dan Sessler, M.D.
Chair, Department of Outcomes Research
Cleveland Clinic

List of Executive Committee Members

Sergio Bustamante, M.D.
Chairperson, Executive Committee
Department of Cardiothoracic Anesthesia

Marc Gillinov, M.D.
Member, DSMB Committee
Department of Cardiothoracic Surgery

Ed Mascha, Ph. D.
Member DSMB Committee
Department of Outcomes Research

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