Does Plasma Volume Replacement with 5% Human Albumin reduce Endothelial Injury and Glycocalyceal Disruption compared with 6% Hydroxyethylstarch (130/0.4) in Patients having Cardiac Surgery?

A substudy of the SHARP clinical trial

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SPECIFIC AIMS

Systemic inflammation caused by exposure to cardiopulmonary bypass or other conditions such as sepsis, ischemia-reperfusion, trauma, major surgery and prolonged hyperglycemia leads to endothelial activation by circulating citokines and subsequent injury to glycocalix and endothelium.[1, 2] Intravascular solutions used for plasma volume replacement, such as human albumin 5%, demonstrate endothelial protective effects which may mitigate the postoperative inflammatory response and consequent endothelial injury.[3, 4] Administration of hydroxyethyl starch (HES) solutions, which have known adverse effects on endothelial function, increases mortality and kidney injury in patients with sepsis [5, 6] perhaps due to aggravation of endothelial and glycocalyceal injury.[7-9] Whether endothelial function is improved in cardiac surgical patients receiving plasma volume resuscitation with 5% human albumin compared with HES solutions, however, is unknown. Our proposed investigation will *test the primary hypothesis* that **postoperative endothelial function,** measured with peripheral arterial tonometry as reactive hyperemia index (RHI) within 2 hours following surgery, **is improved in cardiac surgical patients who are randomized to receive human albumin for plasma volume replacement,** compared with 6% HES solutions. Secondarily, *we hypothesize* that **patients who receive 5% human albumin will have reduced plasma concentrations of molecular biomarkers of glycocalyceal injury (syndecan 1 and endocan)** compared with those who receive 6% HES solutions. *If plasma volume resuscitation with 5% human albumin preserves perioperative endothelial function, kidney dysfunction and other organ injury following cardiac surgery may be reduced.*

Specific aim 1. **To determine whether human albumin 5% improves postoperative endothelial function compared with 6% HES (130/0.4).** Endothelial function will be assessed 2 hours following surgery by measuring RHI using peripheral arterial tonometry (EndoPAT, Itamar Medical Inc., Caesarea, Israel), a well validated tool used in endothelial dysfunction diagnostics and cardiovascular risk stratification.[10-12] We *hypothesize* that patients who receive human albumin 5% for intravascular volume replacement during surgery will have improved endothelial function at 2 hours following surgery compared with those who receive 6% HES (130/0.4).

> Sub-aim 1. Our preliminary data suggest that post-operative endothelial function is poorly correlated with baseline (preoperative) endothelial function. In a subset of patients, we will describe the expected changes that occur in endothelial function during the perioperative period (baseline, early postoperative, 24 hours postoperative. We will assess this for 40 patients (20 per group), at baseline, within 2 hours after surgery, and 24 hours after surgery. We *hypothesise* that there will be a decline in endothelial function from baseline to 2 hours after surgery and no significant difference in function between baseline and at 24 hours after surgery.

Specific aim 2. **To determine whether human albumin 5% reduces plasma biomarkers of endothelial and glycocalyceal damage.** We will measure plasma concentrations of syndecan 1 and endocan at baseline (before surgery), and 1 and 24 hours following surgery. Syndecan 1 and endocan are components of glycocalyx, and increased blood concentrations of these molecules indicate glycocalyceal shedding and endothelial dysfunction.[13, 14] We *hypothesize* that patients who receive human albumin 5% for intravascular volume replacement during surgery will have reduced concentrations of syndecan 1 and endocan at 1 and 24 hours following surgery compared with those who receive 6% HES (130/0.4).

BACKGROUND AND SIGNIFICANCE

Inflammation causes endothelial injury and degradation of the glycocalyx

The vascular endothelium and its proteinaceous coating, the glycocalyx, is omnipresent throughout the circulatory system and plays a key role in preserving vascular integrity and function. The endothelium regulates vascular tone, inhibits intravascular thrombosis and modulates the inflammatory response by modifying the adhesion/migration of leukocytes and other mediators.[8, 14-17] During inflammation induced by cardiopulmonary bypass or sepsis, leukocytes and other inflammatory mediators target the endothelium and glycocalyx causing disruption of vascular endothelial function. Vascular permeability, leukocyte trafficking, and oxidative stress are increased, while vascular tone is reduced. Oxygen and nutrient delivery to cells is impaired and a procoagulant state is promoted.[1, 2, 16, 18]

Leukocyte activation and endothelial adherence contribute to microvascular flow impairment. Vascular hyperpermeability and albuminuria occur, and microthrombi form in the kidney and other organs.[19-22]

Human albumin 5% has beneficial effects on endothelial and glycocalyceal function compared with HES solutions

Human albumin has demonstrated beneficial effects on vascular endothelial and glycocalyceal function in ex vivo models. It prevents fluid extravasation more effectively compared with crystalloid or synthetic HES solutions in an ex vivo heart model, and has been shown to inhibit binding of activated polymorphonuclear leukocytes to bovine aortic endothelial cells. [3] [4] Shear stress-mediated nitric oxide release and coronary vasodilatation is potentiated with administration of human albumin compared with HES solutions.[9] Further, binding of neutrophil-derived myeloperoxidase to bovine aortic endothelial cells, a mediator of multiple oxidative and nitric oxide-consuming reactions, is inhibited by human serum albumin, while it is amplified by HES solutions.[4]Thus the protective characteristics of plasma volume administration with human albumin may reduce endothelial injury in the kidney and other organs, and, ultimately, reduce postoperative organ dysfunction in cardiac surgical patients, especially compared with use of HES solutions.

Endothelial function can be assessed by validated noninvasive techniques

Endothelial function is measured by the magnitude of arterial dilation that occurs following the release of nitric oxide or other endothelium-derived vasoactive substance.[23] Brachial flow-mediated dilation (FMD), a standard measure of endothelial function,[24] is a noninvasive ultrasound-based method that measures the increase in arterial diameter caused by shear stress-mediated endothelial release of nitric oxide. FMD is closely correlated with invasive measures of endothelial function;[25] however, it is technically demanding, affected by the cardiac cycle, and dependent upon the quality of ultrasound images.[24] Peripheral arterial tonometry (PAT) is a sensitive, accurate, and reproducible noninvasive measure of endothelial function. PAT measures endothelium-mediated changes in vascular tone using beat-to-beat plethysmographic recordings of the finger pulse wave amplitude with pneumatic probes at baseline and following release of a 5-min occlusion of the brachial artery (using a standard blood pressure cuff). The endothelium-dependent flow mediated dilation response is manifested as reactive hyperemia which is quantified by the post-occlusion to pre-occlusion ratio. PAT correlates closely with FMD assessed with brachial arterial ultrasound.[12] Use of PAT in patients after cardiac surgery provides a noninvasive reliable and reproducible measure of systemic endothelial function.

Endothelial function can be measured by biomarkers in the blood

Plasma concentration of syndecan 1 serves as a biomarker of endothelial injury and glycocalyx degradation and correlates with serum levels of inflammatory cytokines and mortality in trauma patients.[13] Endocan is another component of the glycocalyx which is released during inflammation in response to tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1), and correlates with severity of sepsis.[14] Assessment of plasma concentrations of syndecan 1 and endocan provide quantifiable measures of perioperative endothelial injury and glycocalyceal disruption.

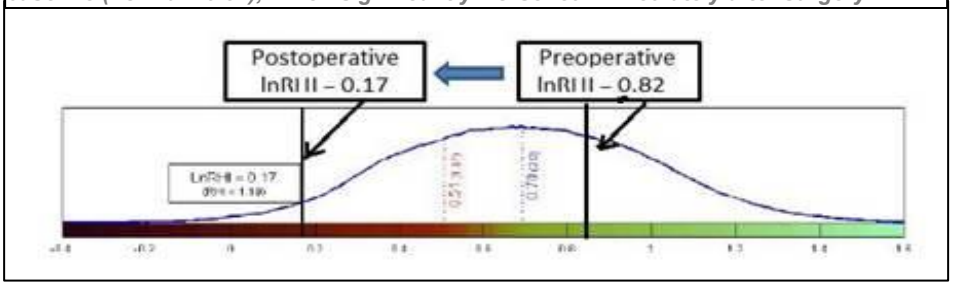
SIGNIFICANCE

Perioperative inflammation and endothelial dysfunction lead to multi-organ system dysfunction following cardiac surgery, including postoperative kidney injury, pulmonary insufficiency, coagulopathy, vasoplegia, hemodynamic instability, and postoperative delirium. Determining whether plasma volume resuscitation with 5% human albumin reduces perioperative endothelial/glycocalyceal dysfunction compared with 6% HES solutions will 1) improve our understanding of the clinical effects of volume replacement solutions, 2) provide evidence to support a mechanism of perioperative and sepsis-associated kidney injury associated with HES solutions, 3) identify potential targets for therapeutic intervention and aid in the development of novel strategies to reduce perioperative and sepsis-induced kidney and other organ injury, 4) contribute to our ultimate goal of reducing postoperative morbidity and mortality in patients who require cardiac surgery.

Preliminary data

We examined the changes in endothelial function in 3 patients having cardiac surgery using peripheral arterial tonometry. All three patients had normal endothelial function (normal lnRHI>0.51) before surgery which worsened significantly after surgery (1 – 4 hours postoperatively).

Figure 1. Baseline (preoperative) and postoperative endothelial reactive hyperemia index, a measure of endothelial function. *This patient had a lnRHI of 0.82 at baseline (normal >0.51), which significantly worsened immediately after surgery.*



EXPERIMENTAL METHODS AND DATA ANALYSIS

Research Design and Methods

This investigation will represent a substudy of a current ongoing clinical trial (SHARP: Voluven vs. Albumin comparing effect of 5% human albumin vs. 6% HES (130/0.4) on postoperative kidney function and coagulation. Patients who consent for SHARP study will be invited for participation in this substudy of endothelial/glycocalyceal function.

Inclusion criteria: Patients aged 40 – 85 years old who signed a written, informed consent for participation in this investigation**,** scheduled for elective aortic valve replacement with or without coronary artery bypass grafting involving nonpulsitile cardiopulmonary bypass, with or without additional minor surgical procedure.

Exclusion criteria: Patients with Raynaud's disease or other disease associated with upper extremity vascular insufficiency**,** and inability to perform EndoPAT exam (inability to lie still for 15 min, or significant finger deformity), patients meeting SHARP exclusion criteria (renal failure with oliguria or anuria not related to hypovolemia, dialysis, preoperative creatinine > 1.6 mg/dL, anticipated deep hypothermic circulatory arrest, known hypersensitivity or allergy to HES, clinical conditions with volume overload e.g., patients in pulmonary edema or congestive heart failure, severe hypernatremia or severe hyperchloremia, intracranial bleeding, pregnant or breast feeding women, critically ill adult patients e.g. patients who are hospitalized in the intensive care unit prior to surgery, severe liver disease, pre-existing coagulopathy).

Randomization

Patients will be randomized to receive either HES 130/0.4 (Voluven) or albumin 5% for treatment of intraoperative acute hypovolemia. (Treatment assignments will be generated using a reproducible algorithm in the PLAN procedure in SAS statistical software. 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.)

Outcomes

Primary outcome is postoperative endothelial function measured by Reactive Hyperemia Index (RHI) using a peripheral arterial tonometer (EndoPAT, Itamar Medical Inc., Caesarea, Israel) within 2 hours following completion of surgery. A subset of patients will also have RHI measured at baseline and 24 hours after surgery.

Secondary outcomes will include blood biomarkers indicative of endothelial/glycocalyceal injury including plasma concentrations of syndecan 1 and endocan measured at baseline (after arterial line

placement), within one hour (± 1 hour) of ICU arrival, and 24 hours (± 2 hours) following completion of surgery (or within 2 hours prior to ICU discharge if patient is discharged in less than 24 hours).

Experimental Protocol

Cardiac surgical patients enrolled in the SHARP study will be randomized to either 5% human albumin or 6% HES on entrance to the operating room. Anesthetic and Surgical management will follow protocol established by SHARP study including administration of a blinded study solution which contains 5% human albumin or 6% HES following separation from cardiopulmonary bypass. Blood will collected for measurement of baseline syndecan 1 and endocan following arterial line and urinary catheter placement. At 1 and 24 hours following surgery completion, blood will be collected for measurement of syndecan 1 and endocan. Within two hours of ICU arrival, PAT will be performed in ICU. In a subset of patients, PAT will be performed preoperatively and at 24 hours after surgery.

Because vasoactive substances may influence EndoPAT measurement, we will collect data on use of all preoperative antihypertensive medications that have direct or indirect effect on smooth muscle relaxation. Cardiac surgery patients often need intraoperative and postoperative inotropic support. Data on use of perioperative vasoactive medications that may influence EndoPAT results including epinephrine, norepinephrine, vasopressin, milrinone, nitroglycerine, nitroprusside, and sedatives such as propofol, dexmedetomidine and others will be recorded and subsequently adjusted for in the analysis. Data on transfusion of packed red blood cells and blood components will be collected from Anesthesia Record Keeping System. Minimum hematocrit (HCT) will be approximately 23% following cardiopulmonary bypass and 21% on cardiopulmonary bypass. Packed blood cell, platelets, fresh frozen plasma and/or cryoprecipitate transfusions may be administered as determined necessary by attending anesthesiologist or surgeon. Any imbalance in blood product transfusion between the two study groups will be adjusted for in analysis.

Data Management

Study Personnel will record data on Case Report Forms. Case report forms will be kept in a locked storage room with access limited to study personnel. Data from Case report forms will be inputted into the study database in REDCap for Data collection and storage. REDCap is a mature, secure web application for building and managing online surveys and databases.The REDCap application allows users to build and manage online surveys and databases quickly and securely, and is currently in production use or development build-status for more than 244,000 projects with over 335,000 users spanning numerous research focus areas across the consortium.

Statistical analysis

Balance of randomized HES and albumin groups on potentially confounding baseline and procedural characteristics will be assessed using absolute standardized difference (ASD), defined as the absolute difference in means, mean ranks, or proportions divided by the pooled standard deviation. We define any variable with ASD > 0.25 as imbalanced and will adjust for such variables in the following analyses. This substudy will be modified intent-to-treat, including all patients who enrolled in SHARP.

Primary analysis

We will estimate the albumin effect on RHI using multivariable linear regression adjusting for imbalanced patient and procedure characteristics. Though we expect RHI to be normally distributed, appropriate transformations will be made if this is not the case (e.g., log transformation). If transformations are not successful, we will use nonparametric methods (i.e., Wilcoxon rank-sum test).

Sub aim analysis

We will estimate the difference in endothelial function from baseline to 2 hours after surgery and baseline to 24 hours after surgery using separate paired t-tests on the subset of 40 patients with these measurements. We will also summarize RHI over time graphically.

Secondary analyses

The albumin effect on each biomarker (i.e., syndecan 1 and endocan) at 1 hour and 24 hours after surgery will be assessed using separate repeated measures linear regression models adjusting for within-subject correlation, baseline biomarkers and imbalanced baseline variables. We will assess the heterogeneity of the treatment effect over time by testing the group-by-time interaction for each model using a criterion of $P < 0.15$. If heterogeneity is detected, we will analyze outcomes separately at each time, using additional Bonferroni correction as necessary. We expect that biomarkers will be lognormally distributed, so we will perform a log transformation and compare groups on ratio of geometric means. An alpha of 0.05 will be used for each set of the primary and sub/secondary analyses, with a significance criterion of 0.05 for the primary analysis and 0.0125 for each sub or secondary analysis (i.e., 0.05/4, Bonferroni). We will complete analyses using SAS version 9.4 or newer (SAS Institute, Cary, NC, USA) and R version 3.2.3 or newer (R Project for Statistical Computing, Vienna, Austria).

Descriptive analyses

Additional morbidity related outcomes will also be reported descriptively for the entire study population. Continuous variables will be reported as mean ± standard deviation or median [interquartile range] as appropriate, while binary outcomes will be reported as N (%).

Sample size analysis

This will be limited to the 85 patients expected to enroll in the SHARP study. Moerland et al found the a mean ± SD of 1.8 ± 0.5 in a healthy study population and 2.9 ± 1.4 in a population with renal injury [M. Moerland, A. J. Kales, L. Schrier, M. G. J. van Dongen, D. Bradnock, and J. Burggraaf, “Evaluation of the EndoPAT as a Tool to Assess Endothelial Function,” International Journal of Vascular Medicine, vol. 2012, Article ID 904141, 8 pages, 2012. doi:10.1155/2012/904141]. We expect similar variability in our cardiac surgery population. Assuming a conservative standard deviation of 1.4, we will have 90% power at the 0.05 significance level to detect an difference of 1.0 or greater in our study population. We will have 65% power to detect a clinically important difference of 0.73 (i.e., 25% of 2.9).

Project Timeline

This project would take 2 years to complete. Institutional Review Board approval would require 2-3 weeks, followed by 1-2 weeks for training and certification of a Research Fellow in proper use of EndoPAT equipment by Itamar Medical representative. Patient enrollment is expected to be complete by January 2018, followed by additional 2-3 months for data interpretation and manuscript preparation.

OTHER SUPPORT

Budget

Total estimated expenses for this proposed research are $25,000. These funds will be used to cover personell costs including 1.2 calendar months effort and 10% salary support for a trained Clinical Research Fellow and 0.6 calendar months effort and 5% salary support for an assigned Outcomes Research Department statistitian. These funds will also cover cost of equipment including EndoPAT probes and labarotory blood testing for endocan and syndecan 1 levels. This project will not involve use of Clinical Research Unit, Lerner Cores, or Biostats.

Future investigation

Perioperative endothelial dysfunction contributes to multi-organ system dysfunction after cardiac surgery. However, investigations examining perioperative endothelial dysfunction have been limited because of the inability to measure and quantify endothelial function. Use of peripheral arterial tonometry provides a unique and effective research tool to measure perioperative endothelial function which can be applied to numerous interventions. The measurement of perioperative biomarkers of endothelial injury

also characterize the consequence of perioperative inflammation in cardiac surgical patients and can be applied to future investigations. Our initial results will faciliate further analysis to determine the contribution of perioperative endothelial function to postoperative morbidity and mortality. Preliminary data gained from this investigation will be used to support future grant applications for investigation of new interventions on postoperative glycocalyceal and endothelial damage measured by peripheral arterial tonometry and other molecular biomarkers. This proposed investigation and future investigations will contribute to our ultimate goal of improving early and long-term patient outcomes following cardiac surgery.

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