Protocol

for

The Optimal Ultrafiltration Protocol in Pediatric Cardiac Surgery: An Observational Cohort Study (*Pilot*)

Principle and Supervising Investigator:

David Horne MD, DCH, FRCSC
Congenital Cardiac Surgeon (IWK & QEII)
Resident Program Director & Clerkship Director (Cardiac Surgery Dalhousie University)
Director: ECLS, IWK Children's Hospital

Division of Cardiovascular Surgery
IWK, 2nd Floor Children's Site, PO Box 9700
Halifax, Nova Scotia, B3K 6R8
Phone: (902) 470-8939
Fax: (902) 470-6616
Email: David.Horne@iwk.nshealth.ca

Participating Centre

Children's Heart Centre, IWK Health Centre, Halifax Nova Scotia

Division of Cardiac Surgery, Department of Surgery, Dalhousie University

David Horne MD (Principle and Supervising Investigator, Surgical Lead, Steering Committee)
Joel Bierer MD (Investigator, Steering Committee)
Suvro Sett MD (Investigator, Safety Committee)
Gregory Hirsch MD (Project Supervisor)

Department of Clinical Perfusion, Nova Scotia Health Authority

Roger Stanzel PhD CPC (Perfusion Co-Lead and Steering Committee)
Mark Henderson CPC CCP (Perfusion Co-Lead and Steering Committee)
Lance Mitchell CPC (Safety Committee)

Department of Critical Care, Department of Pediatrics, Dalhousie University

Neeraj Verma MD (Critical Care Lead and Safety Committee Chair)

Division of Cardiology, Department of Pediatrics, Dalhousie University

Robert Chen MD (Cardiology Lead)

Department of Immunology, Dalhousie University

Jean Marshall PhD (Immunology Lead)
Ian Haidl PhD

IWK Advisory Committee

Jill Hatchett PhD (Trial Design and Statistic Advisor)
Steve Van Ilderstine (Trial Design and Ethics Advisor)

Epidemiology

~~Jong Kim PhD~~
Pantelis Andreou PhD

Table of Contents

1.0 Overview

There have been only a few small studies looking at a limited number of inflammatory marker profiles in children on cardiopulmonary bypass (CPB) with ultrafiltration. Furthermore, these studies were heterogenous in ultrafiltration protocol and patient population. Halifax ZBUF-SMUF uses an ultrafiltration protocol similar to that used at other leading children's heart centers but has not been published or been characterized in terms of inflammatory response. However, ZBUF-SMUF is not by any means considerd an expiremental treatment. Therefore, Phase 1 of the project would be novel in the literature. It would also serve as a proof of principle for Phase 2 RCT.

Study Type: Prospective observational cohort study

Population: Children with congenital heart disease undergoing open heart surgery requiring cardiopulmonary bypass at the IWK.

Sample Size: 40 (2 Subgroups by weight <10kg, 10-30kg subgroup)

Outcome: Inflammatory factors (ET1, TNF-A-α, IL1B, IL6, IL8, C3a, C5a, IL-33/sST2, IL-1Ra, CXCL10, HMGB1, free mitochondria), ~~coagulation factors (fibrinogen, VWF, factor II, VII, IX and X)~~measured in the patient and ultrafiltrate through the operation and up to the first ~~48~~ 24 hours post-op. Post-operative clinical outcomes in the PICU will also be recorded: ventilation time, PICU time, vasoactive-inotrope score (VIS), vasoactive-ventilation-renal score (VVR), blood loss, transfusion rates, stroke use of mechanical circulatory support and death.

Hypothesis: We will describe the pattern of inflammatory markers in a cohort of children following cardiac surgery performed with ZBUF-SMUF ultrafiltration. We hypothesize there will be a correlation between levels of inflammatory markers with post-operative clinical outcomes such as intensive care unit length of stay.

FINER: This study design is feasible as it involves no deviation from current standards of care in children's heart surgery at the IWK. The experts who conduct the clinical care including cardiac surgeon, clinical perfusion, nursing staff, intensivist, cardiology as well as the operating room and PICU infrastructure are all in place and have all signed letters of support for this project. Data collection and analysis protocols have been deemed feasible by the immunology and clinical lead. There are no obvious ethical concerns as all patients receive the same standard of care (see detailed ethical considerations in section 3.0). This study would be novel as Halifax ZBUF-SMUF protocol has not been characterized in terms of inflammatory marker and clinical response.

2.0 Study Objectives

2.1 Publish the Halifax ZBUF-SMUF method.

2.2 Establish the sample collection protocol, including intra- and post-operative cytokines and ~~coagulation factors~~ from blood and ultrafiltrate. Furthermore, establish data collection protocols of post-operative clinical outcomes. This is a pilot for Phase 2 as these data collection processes should be identical.

2.3 Document the characteristics of cytokines~~, coagulation factors~~ related to CPB, and Halifax ZBUF-SMUF.

2.4 Identify novel factors in the ultrafiltrate through discovery immunoanalysis.

2.6 Characterize cardiopulmonary function through VIS, VVR and blood gases lactic acid levels with relation to CPB and ultrafiltration. Furthermore, record post-operative clinical outcomes.

2.7 Identify relationships between inflammatory marker levels and post-operative clinical course.

3.0 ZBUF-SMUF Protocol

ZBUF-SMUF is similar to ultrafiltration protocols described previously. Please see the corresponding review paper for more details. The novel component of the Halifax includes precisely setting the specific ZBUF exchange rate by *Braun Space Infusion Pumps*. This allows for accurate and consistent ultrafiltration which would otherwise be done by "eye-balling" and perfusionist expert opinion.

Terumo HC05 Hemoconcentration and LivaNova S5 Heart Lung Machine are used at the IWK. CPB protocol is as per standard of care as outlined below. This study does not alter the bypass protocol.

3.1 Normalization of Blood Pump Primes. When utilizing blood primes for CPB, ultra filtration is used to maintain a physiologic solution. Primes generally are composed of Plasmalyte A, 0.45% saline, packed RBC and fresh frozen plasma. The amount of crystalloid solutions and ultrafiltration depends on the age of donor blood in the prime and seeks to accomplish physiologic levels electrolytes and blood cells.

3.2 Ultrafiltration During CPB. Fluid volume contained in the pump prime and cardioplegia is removed via ultrafiltration. When excess volume is present in the perfusion circuit during the case, volume is usually removed to maintain maximum hematocrit. There is also a reserve volume in the circuit to be used as clinically indicated.

3.3 Zero-Balance Ultrafiltration (ZBUF). ZBUF is begun soon after initiation of CPB and continues until the patient is weaned from CPB. Between 0-5% of the patient's calculated cardiac output is shunted through the hemoconcentration to participate in ZBUF. Two infusion pumps accurately and simultaneously remove a volume of ultrafiltrate and replace a corresponding volume into the circuit. Replacement solution includes a precise mix of 0.45% NaCl, 0.9% NaCl, plasmalyte A and sodium bicarb.

The optimal exchange rate (ml/kg/hour) is not known. Published ranges include 0 to 80 ml/kg/hour.

3.4 Simplified Modified Ultrafiltration (SMUF). SMUF is used when the patient is weaned from CPB. Between 0-5% of the patient's calculated cardiac output is shunted in a venous-arterial pathway through the hemoconcentrator. The endpoint is when the patient's fluid balance for the case is roughly -5 to -15ml/kg or hematocrit of 40 or hemodynamic instability or volume depletion in the circuit.

4.0 Phase 1 Prospective Cohort Pilot Study

The following points are discussed as according to *the Canadian Institute of Health Research* clinical study guidelines as well as the International Council for Harmonization guide for clinical studies.

4.1 Trial Design

4.1.1 This is a single-center prospective observational trial which will take place at the *Children's Heart Centre, IWK Health Centre.*

4.1.2 Target study size is 40 patients, subgroups by weight (<10kg, 10-30kg). There will be a target of 20 patients per subgroup.

4.1.3 All patients will receive the same standard of care as their condition requires. This study does not alter clinical treatment or management decisions.

4.3 Study Organization

The proposed study will be conducted at the *IWK* in Halifax, Nova Scotia. The usual clinical interdisciplinary team taking part in patient care include members from pediatric cardiology, pediatric cardiac surgery, pediatric cardiac anesthesia, pediatric clinical perfusionists and pediatric intensivists. All these clinical departments have signed a letter of support for this project. The day-to-day coordination and data collection will be conducted by the research coordinator and investigator-sponsor.

4.4 Team Member Role

4.4.1 Cardiac surgery, anesthetist, perfusionist, cardiologist, intensivist, nursing and other specialties will administer care as per usual standards.

4.4.2 Clinician Research staff will be responsible for screening eligible patients, consenting and enrolling patients, collecting biologic samples for analysis, collecting all data points and general trial organization.

4.4.3 Immunology staff and clinician research staff will be responsible for cytokine and coagulation factor measurements and analysis.

4.4.4 The steering committee will consist of cardiac surgeon and perfusion staff and will provide overall supervision of the trial. The steering committee will consist of Joel Bierer, David Horne, Roger Stanzel and Mark Henderson

4.4.5 The Safety Committee will consist of cardiac surgeon, perfusion, intensivist, anesthesia and cardiology staff. The chairperson will not be a surgeon or a perfusionist. The chair will be Neeraj Verma who will be accompanied by Robert Chen, Suvro Sett and Lance Mitchell

4.5 Outcomes and Data Points

4.5.1 Intra-operative cytokines including but not limited to ET1, TNF-A-α, IL1B, IL6, IL8, C3a, C5a, IL-33/sST2, IL-1Ra, CXCL10, HMGB1, free mitochondria. These samples will be taken at the following time points: baseline (prior to sternotomy), post-CPB initiation, every 30-60 minutes while on CPB/ZBUF, post cardioplegia volume reduction, pre-SMUF, post-SMUF, 6 hours post-SMUF, 12 hours post-SMUF, 24 hours post-SMUF and ~~48 hours post-SMUF~~. Samples taken while on CPB will be paired with samples of ultrafiltrate in the line as well as the reservoir.

~~**4.5.3** Intra-operative coagulation factors including but not limited to fibrinogen, VWF, factor II, VII, IX and X. These samples will be taken at the following time points: baseline (prior to sternotomy), post-CPB initiation, every 30-60 minutes while on CPB/ZBUF, post cardioplegia volume reduction, pre-SMUF, post-SMUF, post-SMUF, 6 hours post-SMUF, 12 hours post-SMUF, 24 hours post-SMUF and 48 hours post-SMUF. Samples taken while on CPB will be paired with samples of ultrafiltrate in the line as well as the reservoir.~~

4.5.3 Intra-operative blood gases will be taken at regular time intervals. These samples will be taken at the following time points: baseline (prior to sternotomy), post-CPB initiation, every 30-60 minutes while on CPB/ZBUF, post cardioplegia volume reduction, pre-SMUF, post-SMUF, post-SMUF, 6 hours post-SMUF, 12 hours post-SMUF, 24

hours post-SMUF and ~~48 hours post-SMUF~~. These blood gases are considered standard of care for "goal-directed" perfusion and therefore are part of normal practice. Although these results will be recorded, they do not constitute extra blood sampling for research purposes.

4.5.4 Complete blood count (CBC), Activated Clotting Time (ACT), hemolysis panel (haptoglobin, plasma free hemoglobin, lactate dehydrogenase and indirect bilirubin) after weaned from bypass in the OR.

4.5.5 Post-operative clinical outcomes including regular (per PICU protocol) blood gases, chest tube output (ml/kg), transfusion requirements (ml/kg), inotrope use (VIS), vasoactive-ventilation-renal score (VVR), ventilation time (hours), ICU stay (days), stroke or neurologic injury, use of mechanical circulatory support and death.

4.6 Eligibility Criteria

4.6.1 All congenital cardiac patients that have been consented for a planned cardiac surgery procedure requiring cardiopulmonary bypass at the IWK.

4.6.2 Patient or family consent to participate in the study.

4.7 Exclusion Criteria

4.7.1 Patient or family refusal to participate.

4.7.2 No planned use of cardiopulmonary bypass.

4.7.3 Known hematologic abnormality such as sickle cell anemia, thalassemia, hemophilia A or B, von Willebrand disease or other.

4.7.4 Known genetic syndrome with multi-organ abnormalities and immune dysfunction such as DiGeorge Syndrome, Trisomy 18 or 13, Noonan syndrome.

4.7.5 Known immunodeficiency syndrome or bone marrow pathology.

4.7.6 Severe liver disease with abnormal synthetic liver function tests.

4.8 Recruitment

4.8.1 Patients will be recruited after being accepted by Pediatric Cardiac Surgery and Cardiology specialists at the IWK. The research coordinator, or investigator-sponsor if the research coordinator is unavailable, will approach patients and their families to assess interest in participating in the study.

4.8.2 Approximately 80 Pediatric cardiac operations requiring cardiopulmonary bypass occur each year at the IWK. It will take roughly ~~3-6~~ 8 months to accrue enough patients.

4.8.3 Recruitment will take place at the IWK only, one single center.

4.8.4 All data required for a consort diagram will be collected. Please see the screening forms.

4.9 Consent

4.9.1 This study will require informed consent as well will have a standard operation procedure for obtaining consent. Please see Consent Package and Standard Operating Procedure documents.

4.9.2 Informed consent will be obtained by the research coordinator or the investigator-sponsor if the research coordinator is unavailable.

4.9.3 Many patients in this population lack capacity and therefore their substitute decision makers, often their parents or guardians, will be approached. This person themselves will have to have the capacity to make such a decision.

4.9.4 All patients enrolled in the study will have documentation of trial participation in the chart.

4.10 Initial Screening

All potentially eligible patients will be recorded (study logbook) by MRN. No additional information demographic will be gathered at this time. A congenital heart disease diagnosis must be established by the referring physician before the patient will be scheduled for an eligibility interview. All patients with scheduled cardiac operations requiring cardiopulmonary bypass will be examined taking into consideration the eligibility and exclusion criteria. This will be done at the time of pre-operative consultation. Please see standard operating procedures for more details.

4.11 Eligibility Visit and Pre-operative Assessment

Following surgical consultations, potential candidates will be seen by preferentially a research coordinator, or the principal investigator if research coordinator unavailable, to be assessed for trial participation and discuss possible enrollment. Please see standard operating procedure document.

The following will be obtained at the eligibility visit: (1) written informed consent (2) history of

relevant conditions and current medical condition (3) review of indicated investigations which may include echocardiograms, chest radiographs; computed tomograms of chest and upper abdomen, pulmonary function tests, and arterial blood gases at rest. Indicated labs will be reviewed which might include CBC and differential, extended electrolytes, renal and liver function will also be reviewed. STS-EACTS score will also be recorded. Please see standard operating procedure document.

All patients screened or interviewed will be kept in a log. Information of the log will include the date of the interview, MRN, diagnosis, reason why the patient was not enrolled or confirmation of enrollment. Please see the standard operating procedure.

4.12 Overview of Treatment Plan

All patients will undergo standard of care cardiac procedures, use of cardiopulmonary bypass and ZBUF-SMUF. They will receive the standard of care in the PICU post-op. This is according to institutional protocols and standards. This study has no impact on therapies, nor does it lead to any divergence from the standard of care.

4.13 Standardization of Technique

All participating surgeons and perfusionists have agreed to follow a similar procedure as currently practiced at the IWK. The patient's well-being and safety will always receive top priority and will be left to the individual surgeon's discretion and clinical judgment in the event of unexpected deterioration or emergency.

4.14 Surgical and Ultrafiltration Technique

All cardiac surgery procedures will be done as usual by cardiac surgery staff. This includes routine cannulation for cardiopulmonary bypass. Ultrafiltration protocol has been described in section 2.0. All children will have ZBUF exchange of -5ml/kg/hour. This is the current standard at the IWK. Cardiopulmonary bypass practices are per standard of care.

4.15 Surgical Quality Assurance

As per institutional protocol, any unexpected morbidity or mortality will be reviewed by the Safety Committee as previously described. This is an observational study and it is very unlikely to cause any morbidity or mortality.

4.16 Data Collection

4.16.1 The follow-up and data collection will be conducted by the principal investigator each during the cardiac procedure and each day until PICU discharge.

4.16.2 ET1, TNF-A-α, IL1β, IL6, IL8, C3a, C5a, IL-33/sST2, IL-1Ra, CXCL10, HMGB1, free mitochondria levels will be measured through assays by the immunology department. These venous samples will be taken at the following time points: baseline (prior to sternotomy), post-CPB initiation, every 30-60 minutes while on CPB/ZBUF, post cardioplegia volume reduction, pre-SMUF, post-SMUF, 6 hours post-SMUF, 12 hours post-SMUF, 24 hours post-SMUF ~~and 48 hours post-SMUF~~. Samples taken while on CPB will be paired with samples of ultrafiltrate. Samples will be in EDTA tubes. All blood samples will be centrifuged at 400g for 10 minutes at room temperature, have the plasma extracted which will be further centrifuged at 2400g for 20 minutes at room temperature. The resulting supernatant will be flash frozen with liquid nitrogen. All samples will be stored in a freezer at -80C until Luminex Immunoassay analysis when all study samples are collected. Interim quality control analysis will occur on a limited number of samples throughout the study.

The analysis will be performed in a tapered fashion. For example, the first 10 patients will be tested for a large number of markers. Using those results we will selectively taper down to monitor the active cytokines. For example, if cytokine X is not detected in the first group of patients, it will not be tested in the next group and will very likely be excluded in Phase II.

~~**4.16.3** Fibrinogen, VWF, factor II, VII, IX and X levels will be measured through assays by the immunology department. These samples will be taken at the following time points: baseline (prior to sternotomy), post-CPB initiation, every 30-60 minutes while on CPB/ZBUF, post cardioplegia volume reduction, pre-SMUF, post-SMUF, 6 hours post-SMUF, 12 hours post-SMUF, 24 hours post-SMUF and 48 hours post-SMUF. Samples taken while on CPB will be paired with samples of ultrafiltrate. These samples will be processed and stored and sent to "In Common Laboratories" for analysis.~~

4.16.5 Routine arterial blood gas, CBC, ACT and hemolysis analysis will also be taken at time points to match cytokine and coagulation factor measurements. These blood tests are routinely done as the standard of care to achieve "goal-directed" perfusion. Although the values will be recorded, they do not constitute experimental or extra blood sampling.

4.16.6 Post-operative clinical outcomes including regular (per PICU protocol) blood gases at 6 hours post-SMUF, 12 hours post-SMUF, 24 hours post-SMUF and 48 hours post-SMUF, CT output (ml/kg), transfusion requirements (ml/kg), inotrope use (VIS), ventilation-vasoactive-renal score (VVR), ventilation time (hours), ICU stay (days), stroke or neurologic injury, use of mechanical circulatory support and death will be collected by daily chart review. Adverse events will be collected as defined by the STS.

4.16.8 Blood Draw Schedule. The first 2-4 participants will have simultaneous

ultrafiltrate samples from the line and reservoir to be analyzed prior to the rest of the group. This is to determine whether the rest of the study will be sampled from the line or direct from the reservoir.

	Sample Collection Checklist																			
	Pre-Op	Pre-Stern	(Blood Prime)	Post-Stern	On-CPB	CPB-30	CPB-60	CPB-90	CPB-120	CPB-150	CPB-180	CPB-240	CPB-300	CPB-360	CPB-420	Pre-SMUF	Post-SMUF 0	ICU Adm	Post-12	Post-24
Time	■																			
Serum Immuno	■																			
Ultrafilt Line Immuno	■	■																■	■	■
Ultrafilt Res Immuno	■	■																■	■	■
Art Gas	■																			
Ult Line Gas	■	■																■	■	■
CBC		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■				
INR		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■			
PTT		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■			
CR		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■			
Fibrino		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■
Hapto		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■
LDH		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■
Indirect Bili		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■

Each venous draw for immunologic assay will be 1ml. All other blood draws are considered as part of the standard of care and would not constitute extra blood sampling for research purposes.

4.16.9 Biologic Sample Processing. Blood will be obtained from consenting patients in EDTA Eppendorf tubes 1.5ml. The collected blood will be centrifuged at 400g for 10 minutes at room temperature. The resulting plasma is further centrifuged at 2400g for 20 minutes at room temperature to remove platelets. The plasma samples will be aliquoted in appropriate volumes and flash frozen before storing at -80°C. All samples will be labeled to permit future identification while ensuring the anonymous nature of all information. All samples, prior to processing will be kept in a locked freezer in the OR hallway that has been allocated to the process by OR Facilities and Nursing.

Multiplex Luminex analysis will be performed with a Bio-Rad Bio-Plex 200 system. Panels of magnetic Luminex analyte beads are available from several commercial sources and will be chosen to optimize analysis of the collected patient samples. The aliquoted samples will be thawed and analyzed according to the manufacturer's instructions. Briefly, this entails diluting the plasma samples and analyte standards, adding the samples to the chosen combination of Luminex beads, and adding the samples to a 96-well plate. Following a two hour incubation at room temperature with shaking at 800 rpm, the 96-well plate is washed four times with an automatic plate washer (Bio-Rad, Bio-Plex Pro II). A cocktail of biotinylated secondary antibodies for the analytes is added to each well and incubated again at room temperature for one hour with shaking at 800 rpm. The plate is washed again four times before adding diluted streptavidin-PE to all of the wells. The plate is incubated again at room temperature for 30 minutes with shaking at 800 rpm before a final set of four washes. The beads are resuspended in the buffer to allow reading of each individual well in the Bio-Plex 200. The concentration of each analyte for each sample will be calculated based on the standard curves generated from

each plate.

4.16.10 Blood Sample Analysis. Immunoanalysis will be completed at Dalhousie's Immunology department under Dr. Jean Marshall's Lab. Following successful analysis, any remaining blood sample will be disposed of in a biohazard fashion. Coagulation analysis will be completed through a third party "In Common Laboratory", any remaining blood sample will be disposed of in a biohazard fashion. For both of these analyses, samples will be coded and de-identified so no personal health information is transferred. Material transfer agreements will be in place prior to these analyses.

4.17 Adverse Events and Safety

4.17.1 This is an observational study only. There is no intervention and therefore unlikely to cause significant morbidity or mortality in the patient population.

4.17.2 The most responsible physician will be responsible for the safety or participants under his or her care. The Safety Committee will have primary responsibility for the monitoring of study data for adverse trends and morbidity. This will happen every 3 months unless more urgently indicated. Please see the standard operating procedure.

4.17.2 Several illnesses and major events may befall patients during the study. As usual, cardiac surgeon, anesthetists, PICU staff and cardiologist are free to treat each patient according to their best judgment.

The post-operative clinical course of congenital heart disease is one in which a significant number of patients treated with standard therapy will deteriorate or die. Significant deterioration may be in the form of worsening symptoms of heart failure, respiratory failure, cyanosis, multi-system failure or post-operative complications. The treating specialist and team should always use the most appropriate management that is in the best interest of the patient.

4.18 Post-operative Therapy

All patients should receive full care as indicated and according to the patient's goals of care. This might include prolonged intubation and ventilation, mechanical support, inotrope use, antibiotics, blood transfusion, chest tubes, central lines and other intensive care medications and procedures. As there is no intervention in this observational study, this study has no impact on post-operative clinical care or course.

4.19 Confidentiality

4.19.1 The confidentiality of all participants will be protected both at the study site and the Study Coordinating and Methods Center (Room K2330) within the IWK Children's

Heart Center. Completed data collection forms will receive the same protection as other medical records. Reporting of study results will not identify individual patients. Only the dedicated clinician researcher will collect and access these records.

4.19.2 Personal health information will be collected. Electronic data will only be kept on the principal investigator's secure IWK servers only. H:\IWK\Research\DOS-PVSRG\Horne\ZBUF-SMUF. Data on paper will be kept in a locked research dedicated area within the IWK Children's Heart Center (Room K2330). Please see standard operating procedures

4.19.3 Personal health information collected may include demographics (such as age, gender, MRN), medical history, laboratory or imaging results, post-operative outcomes previously identified as well as assay results from biologic samples. This will be disclosed on the study consent form. This information will be collected from IWK clinical and health records. This collected information will be kept secured as previously described. The collection of this personal health data is important for the study is characteristics of this cohort need to be described to make the research results meaningful. Furthermore, many of the outcomes are clinically based and need to be extracted from daily charts.

4.19.4 Patients will be de-identified. There will be one master electronic file kept on IWK server and one hard copy kept in a locked research dedicated area within the IWK (Room K2330). All other data sources or copies will be completely de-identified by assigning each patient a single- or double-digit number. This will be documented and correlated with the previously described master file kept on secure IWK servers.

4.19.5 Patient information will not be sent off-site or transferred to third party.

4.19.6 A permanent master electronic file will be kept on IWK server for the foreseeable future. All paper file will be disposed of by confidential shredding after one year of study completion.

4.20 Sample Size Determination

The sample size is 40. This will give roughly 20 participants in each of the 2 weight subgroups (<10kg, 10-30kg). This number was chosen by reviewing similar observational trials which often have between 10-40 participants. There is no sample size calculation as this is purely an observational pilot trial.

4.21 Statistical Analysis

4.21.1 A descriptive analysis will be done which characterizes the changes of inflammatory marker levels with time. Both an absolute change as well as change from baseline will be used. This is a time series analysis.

4.21.2 Subgroup analysis will divide patients into ~~4~~ 2 subgroups by weight (<10kg and >10kg) ~~(<5kg, 5-10kg, 10-15kg, 15-30kg)~~. A direct comparison will be conducted between subgroups in terms of inflammatory levels change from baseline.

4.21.3 A correlation analysis will look for an association between ventilation time/VIS/VVR with inflammatory marker elevation.

4.21.4 There will be subgroup analysis between males and females.

4.22 Publications

The main publications for the trial will be in the names of all full collaborating investigators. Subsidiary papers will be authored by study investigators.

4.23 Study Timetable

January 2019 – December 2019	Study planning, ethics, funding.
January 2020 – December 2020	Phase 1 Study

4.24 Limitations, Potential Pitfalls and Strategies

4.24.1 Selection bias will be kept to an absolute minimum as all eligible patients will be consulted by the research staff. Exclusion criteria have been put into place as to not confound the results by including patients with hematologic or coagulation abnormalities.

4.24.2 New information may become available for superior ultrafiltration methods or intra-operative practices. This may make it unethical to continue with the protocol. It would be unusual for clinical practice to drastically change based on the results of one or maybe two trials. Confirming evidence is always needed to support the results. We are not aware of any trial currently evaluating different ZBUF rates.

4.24.3 Due to the large number of biological sample and analyses proposed, there could be a risk of unprocessed samples which might require longer patient enrollment to reach the sample size target.

4.24.4 Perfusion during cardiac surgery sometimes requires deviation from a standardized protocol. The safety and well-being of the patient will always be prioritized over the study protocol. This deviation, however, could confound the results.

4.25 Health Economic Considerations

There is no health economic measure in this trial. This study does not focus on how health care is consumed or administered. It focuses on a very specific and specialized technique.

4.26 Quality of Life

There is no quality of life measure in this trial. Quality of life would be difficult to measure in the high acuity environment of pediatric cardiac surgery. The focus of this study is to identify techniques that reduce inflammation, morbidity and mortality so that perhaps this patient may

more quickly return home with their families in good health.

4.27 Patient Involvement in Trial Development

The study is planned to have patient advocates, the process is ongoing to find interested individuals. These are patients and families who have undergone the experience of pediatric cardiac surgery. Their responsibilities would involve discussing the project design with researchers and giving any important input from the patient's perspective. They would also be available to discuss the study with any potential participants who wish to learn more about the study.

4.28 Genetic Analyses

There are no genetic analyses or samples in this trial.

4.29 International Collaboration

Collaboration is essential to the progress of technology, science and health care. Should there be interest from other centers to participate in this research project, they would be warmly welcomed. This could be done by networking at research conferences and direct contact to investigators. New collaboration would be reported to the ethics board. At the current time, there are no international collaborators.

4.30 Patient Information Sheet

A patient information sheet has been designed and included in the Consent Package.

4.31 Ethical Considerations

4.31.1 This study is an all-comer study and does not target any vulnerable population or community. There are no exclusion criteria that violate the principle of inclusiveness.

4.31.2 This study in no way will lead to deviation in standard of care.

4.31.3 This study in no way involves deception.

4.31.4 There is nothing in the protocol that limits the ability to notify patients or other investigators or risks or adverse events identified in the study. In

fact, the study protocol requires they be documented and brought forward.

4.31.5 Should any patient, participant or family member become distressed due to study participation, there is a dedicated investigator-sponsor who is readily available for counsel. Consent for participation can also be withdrawn. Please see standard operation procedures.