Targeting Normoxia in Neonates With Cyanotic Congenital Heart Disease in the Intra-operative and Immediate Post-operative Period (T-NOX)

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Title: Targeting Normoxia in Neonates with Cyanotic Congenital Heart Disease in the Intra-operative and Immediate Post-operative Period (T-NOX)

Background:

Infants who undergo cardiac surgery with cardiopulmonary bypass (CPB) are at significant risk of morbidity and mortality associated with end-organ injury and dysfunction. The etiology for end-organ injury and dysfunction after heart surgery is multi-factorial and likely related to intraoperative techniques, residual cardiac lesions, a systemic inflammatory response, and several patient-specific factors such as age, weight, level of preoperative support, genetic abnormalities, cardiac diagnosis and type of surgical procedure. Not surprisingly, there is wide institutional variation in outcomes after cardiac surgery in infants and young children.

During open heart surgery with CPB, the myocardium is exposed to a period of ischemia and reperfusion, which is associated with mitochondrial dysfunction and oxidant stress. Despite the well-described role of oxidant stress in ischemia-reperfusion injury, the current practice at many centers is to administer supra-physiologic oxygen levels (hyperoxia) to infants through the cardiopulmonary bypass circuit and mechanical ventilator, which could theoretically exacerbate oxidant stress and reperfusion injury. These detrimental effects of hyperoxia may be more pronounced in neonates and infants who are known to have immature anti-oxidant defenses compared to older children and adults[1], and further influenced by preoperative cyanosis.

There is mounting experimental evidence and several clinical studies that support the concept that hyperoxia (versus normoxia) is associated with adverse outcomes after ischemia. Animal models of CPB and cardioplegic arrest show that hyperoxic myocardial reperfusion is associated with increased oxidant stress, inflammation, and organ injury compared with normoxic reperfusion[2]. Furthermore, hyperoxia during and after cardiopulmonary resuscitation has been associated with an increased risk of mortality in both children and adults[3-6]. Lastly, in pediatric patients requiring veno-arterial extracorporeal membrane oxygenation, hyperoxia was associated with worse clinical outcomes[7, 8].

There are a few comparative trials of hyperoxic versus controlled normoxic reperfusion following CPB in adults and children with mixed results. In two adult studies, there were no observed clinical differences between the different reperfusion strategies [9, 10], however Ihnken and colleagues showed a significant increase in pulmonary function and evidence for decreased oxidant stress biomarkers in patients exposed to normoxia compared to hyperoxia [11]. In older children undergoing cardiac surgery, exposure to lower oxygen levels during cardiopulmonary bypass produced a significant reduction in oxidant stress biomarkers [12-14], biomarkers of brain injury [13, 15], and length of mechanical ventilation [14, 16]. In single ventricle patients, normoxic reperfusion during CPB also improved long-term survival, systemic ventricular function, and heart failure classification [17]. A summary of the studies in adults and children is shown in Table 1.

Table 1: Summary of studies in adults and children comparing hyperoxic vs normoxic reperfusion following cardiopulmonary bypass.

Authors	Date	Age	Sample Size	Trial Type	Description	Results
Smit et al	2016	Adult (median 67 (IQR 63-71)	57	Randomized controlled trial of normoxia (PaO2 130-150) vs Hyperoxia (PaO2 200-220) in patients undergoing coronary artery bypass grafting	No adverse events or mortality reported	No difference in biomarkers of myocardial injury, oxidative stress, end-organ function. No difference measured cardiac index or systemic vascular resistance
McGuiness et al	2016	Adult (20-90 years)	298	Randomized controlled trial of normoxia on CPB (PaO2 75-90) vs standard of care (PaO2 170-200)	No adverse events or side effects reported. 9 deaths total (4 in control group and 5 in intervention group)	No difference in occurrence of renal dysfunction (primary outcome). No differences in biomarkers or other clinical outcomes (ICU stay, ventilator time, mortality)
Caputo et al	2014	Pediatric (29 days to 17 years)	69	Cyanotic patients randomized to normoxia (50-100) vs standard of care (160-170)	No adverse events and no deaths <30 days after surgery reported	No difference in clinical outcomes (length of stay, ventilator time). There was a significant decrease in heart failure symptoms (mean follow-up time of 7 years) in patients exposed to normoxia compared to hyperoxia. Cardiac function as measured by echo was also significantly different in the normoxia group (more patients with EF>50%). Statistically significant decrease in biomarkers for oxidative stress, myocardial, cerebral, hepatic dysfunction, stress/inflammatory response.
Babu et al	2012	Pediatric (9 months – 5 years)	29	Randomized controlled trial of cyanotic patients (sat<85%) comparing initiating CPB with FiO2 of 21% (with titration up to 60% after 5 minutes) vs FiO2 of 60%	No major adverse events reported. Two deaths in each group.	Ventilation time was significantly lower in the normoxia group with no other clinical differences seen. Statistically significant decrease in myocardial injury as measured by CK-MB in the normoxia group.
Caputo et al	2009	Pediatric (6-49 months)	67	Cyanotic patients were randomized to hyperoxia (150-180) vs normoxia (50-80) on CPB	No adverse events reported and no deaths reported	No difference in clinical outcomes (ventilator times or ICU stay). Statistically significant decrease in biomarkers for oxidative stress, myocardial damage, cerebral and hepatic injury in the normoxia group compared to hyperoxia.
Bulutcu et al	2002	Pediatric (3 months – 5 years)	14	Cyanotic patients (sat<85%) were randomly allocated to PaO2 of 300-350 or PaO2 of 90-110	No safety issues reported and no deaths.	No difference in intubation times or ICU stay. Statistically significant decrease in oxidative stress biomarker (MDA) from a myocardial tissue biopsy and decrease in pro-inflammatory cytokines (TNF-a and IL-6) in the normoxia group compared to hyperoxia group
Matheis et al	2000	Pediatric (5 days to 15 years)	21	Cyanotic patients allocated (non-randomized) to normoxia on CPB (PaO2<150) vs hyperoxia (PaO2>150)	No adverse events reported. Two deaths reported (<30 days of surgery) both in hyperoxia group. One death in an infant in the normoxia group was reported at 34 days after surgery	Increased release of S100 biomarker (neurologic injury) in cyanotic patients exposed to hyperoxia compared to normoxia
Ihnken et al	1998	Adult (mean 61 years)	40	Randomized to hyperoxia on CPB (PaO2 400) vs normoxia (PaO2 140)	No adverse events reported	Statistically significant increase in oxidative stress biomarkers and myocardial damage in hyperoxic group compared to normoxia. No differences in clinical outcomes of ICU/hospital stay. Statistically significant decrease in lung function measured by PFTs in hyperoxia group 5 days post-operatively

Overall, pediatric clinical and pre-clinical experimental studies suggest that there is a likely benefit to normoxic and controlled re-oxygenation during cardiac surgery. However, there are several limitations to the published data including single center studies enrolling a wide age range, heterogeneous patient populations encompassing a wide variety of cardiac diagnoses and surgical procedures, and an inconsistent approach to preventing early exposure to supra-physiologic oxygen levels. Furthermore, results from an informal survey of other large pediatric cardiac surgery centers (Table 2) and survey data from 39 centers from a large multi-institutional registry (NPC-QIC) (Figure 1) indicates that most centers utilize a supra-physiologic oxygen management strategy and that there is significant practice variation with regards to goal oxygen levels during cardiopulmonary bypass. Therefore, the goal of this proposed study is to investigate the use of normoxia or near-physiologic oxygen levels before, during, and after cardiopulmonary bypass in a well-defined, high-risk cyanotic neonatal population undergoing a select number of surgeries. The study will be a pilot single center study to show efficacy and safety with the eventual goal of a larger multi-center study that will be powered to show a true difference in clinical outcomes.

Table 2: Survey results from large pediatric cardiac surgery centers reporting PaO2 management on bypass practice.

PHN Center	Practice with regards to PaO2 management on Bypass
Michigan	All patients: 200-300
MUSC	Cyanotic patients at baseline: Initiate at 70-100 and 150 during rewarming. All other patients: 150-250
Emory	All patients: 250-300
Boston Children's	All patients: >500
CHOP	All patients: 200-250
Texas Children's	All patients: Goal 150-250
Sick Kids Toronto	All patients: Goal 120-150 on bypass Cyanotic patients: Initiate bypass with PaO2<100 and then increase to goal
Cincinnati Children's	Cyanotic patients at baseline: Initiate bypass at 21% FiO2 and then increase to 200-250. Decrease to 80-120 prior to cross clamp removal and then goal of 200-250 All other patients: Maintain >200, decrease to 80-120 just prior to cross clamp removal and then 200-250 afterwards

ANSWER CHOICES	RESPONSES	
Match patient's baseline	0.00%	0
< 30	0.00%	0
30 - 70	0.00%	0
71 - 120	15.38%	6
121 - 200	30.77%	12
201 - 300	43.59%	17
301 - 400	2.56%	1
> 400	7.69%	3
TOTAL		39

Figure 1: Survey Data results of NPC-QIC Perfusion Practices: Goal PaO2 while on cardiopulmonary bypass.

Specific Aims:

1. To assess oxidative stress and end organ injury in the normoxia versus supra-physiologic oxygen groups.

   Hypothesis 1a: Normoxia will be associated with reduced oxidative stress as measured by thiobarbituric acid reactive substances (TBARS), a marker of lipid peroxidation. We also anticipate reductions in other measures including protein carbonyl, 8-isoprostane, and whole blood reduction-oxidation potential.

   Hypothesis 1b: End organ injury will be reduced as assessed by biomarkers of renal (Neutrophil gelatinase-associated lipocalin), neurologic (Glial fibrillary acidic protein, Tau), liver (Intestinal fatty-acid binding protein), vascular (Angiopoietin 2), and myocardial injury (Troponin I) as well as immune system activation (IL-6, IL-10).

2. To evaluate the safety profile of a strategy of normoxia versus supra-physiologic oxygen.

   Hypothesis: Observed major adverse events including in-hospital mortality, cardiac arrest, need for mechanical circulatory support, seizures, and dialysis will be similar or lower in the normoxia group.

3. To investigate optimal endpoints in the study of the clinical impact of normoxia.

We will assess clinical endpoints that may be reflective of a reduction in oxidative stress to understand effect sizes and inform the design of subsequent trials. These endpoints will include post-operative length of stay, days alive and out of the intensive care unit (ICU) at 30 days after surgery, a composite outcome of major adverse events as described above, and a global rank score.

Study Design:

Participants:

Single center (University of Michigan) randomized pilot study in infants less than 30 days of age undergoing heart surgery with cardiopulmonary bypass. The trial will be unblinded with respect to knowledge of treatment assignment as the majority of clinical personnel will have knowledge of the cardiopulmonary bypass strategy based on blood gases and operative documents.

Inclusion Criteria:

- Age less than 30 days of age at time of surgery with need for cardiopulmonary bypass with cardioplegic arrest (with or without deep hypothermic circulatory arrest)
- Diagnosis with cyanosis at baseline (pre-operative PaO2 of less than 50mmHG) due to:
    - Complete admixture lesion (example: hypoplastic left heart syndrome, total anomalous pulmonary venous return, truncus arteriosus, pulmonary atresia with VSD)
    - Transposition physiology (example: D-Transposition of the great arteries or Double outlet right ventricle with subpulmonary VSD)
    - Right-to-left shunt (example: Tetralogy of Fallot, double outlet right ventricle with suboaortic VSD and pulmonary stenosis)

Exclusion Criteria:

- Corrected gestation at time of surgery less than 37 weeks
- Prior cardiac arrest
- Current or prior history of ECMO support
- Current or prior history of needing renal replacement therapy with dialysis
- Prior cardiac surgery requiring cardiopulmonary bypass
- Diagnosis of Ebstein's Anomaly
- Known genetic syndrome other than Trisomy 21 or DiGeorge Syndrome

Subject Availability:

Based on historical data, the number of patients that fit the inclusion criteria would be about 40-50 patients per year in this single center study. A sample size of 21 patients per treatment group (42 total patients) will be enrolled. With a 50-67% consent rate, the study duration will be approximately 24 months.

Trial Enrollment and Randomization:

Infants will be randomized into two groups:

1. Normoxia Group (with controlled re-oxygenation):
    a. Goal PaO2 on cardiopulmonary bypass of 60-100 mm Hg using lower FiO2 (blended sweep gas) via oxygenator
    b. Post-bypass, goal of PaO2 <100 mm Hg by anesthesia and in ICU via oxygen titration via mechanical ventilator for 24 hours post-op.
2. Standard of Care Group: Current standard of care

Randomization will be performed in random permuted block sizes of 2 or 4 and stratified by "Stage I Palliation" (Norwood arch reconstruction with shunt placement [Right ventricle-to-pulmonary artery shunt, modified Blalock-Taussig shunt, or central aortopulmonary shunt]) in each treatment group. Patients undergoing Stage I palliation are known to have higher rates of complications and mortality at baseline and therefore the stratification will be done to control for this in the randomization.

Study Protocol:

Please see Figure 2 for a summary of the study protocol.

1. Preoperative:

Patients that meet the defined inclusion and exclusion criteria will be identified and the families will be approached for consent prior to the scheduled surgery.

Patients that are enrolled in the study will have the following pre-operative studies:

- Head ultrasound (not currently standard of care but will be used to evaluate for any brain injury in each group)
- Renal function and liver function panel (within 24 hours prior to surgery)

2. Operating Room:

Patients will be brought to the operating room in a standard fashion. Anesthesia will induce general anesthesia per protocol and secure an airway (if needed) and place invasive lines. Patients will have labs drawn (baseline biomarkers of oxidative stress, troponin level, and Redox potential) and an arterial blood gas.

All patients will have a median sternotomy and be cannulated onto cardiopulmonary bypass via standard procedures at the discretion of the surgery team.

3. Initiation of Cardiopulmonary Bypass:

The cardiopulmonary bypass circuits will be set up and primed per standard institutional protocols.

"Normoxia" Group: Prior to cannulation, a blood gas will be drawn from the cardiopulmonary bypass circuit and correlated to the CDI (in-line PaO2 monitor). The FiO_2 of the oxygenator will then be titrated to achieve a PaO_2 of the circuit and CDI of 60-100 mm Hg. After cannulation, frequent blood gases will be checked per protocol and correlated with the CDI to maintain a PaO_2 of 60-100 mm Hg.

"Standard of Care" Group: After cannulation, frequent blood gases will be checked per protocol on bypass and correlated with the CDI to maintain a PaO_2 of 200-300 per standard practice.

4. Weaning of Cardiopulmonary Bypass:

At the end of re-warming, a blood sample will be obtained for appropriate studies.

"Normoxia" Group: As cardiopulmonary bypass is being weaned, anesthesia will initiate mechanical ventilation with an FiO2 of 50% or less (unless clinically necessary) to achieve oxygen saturation and PaO_2 goals that fit within the expected range for the patient's physiology:

1. Single ventricle patients (PaO_2:35-45 and oxygen saturation 75%-85%)
2. Two ventricle patients (PaO_2: 60-100 and oxygen saturation >92%)

Measures of oxygen delivery such as NIRS monitoring, mixed venous oxygen saturations, and lactate measurements will be used to verify appropriate oxygen delivery.

"Standard of Care" Group: As cardiopulmonary bypass is being weaned, anesthesia will initiate mechanical ventilation per standard protocols. Ventilation will be continued in the ICU and adjusted per standard goals per the intensivist.

5. Post-Operative Course:

After transfer to the PCTU from the OR, the goals as stated above based on the patient's physiology will be maintained for 24 hours after the surgery per the goals per group and physiology.

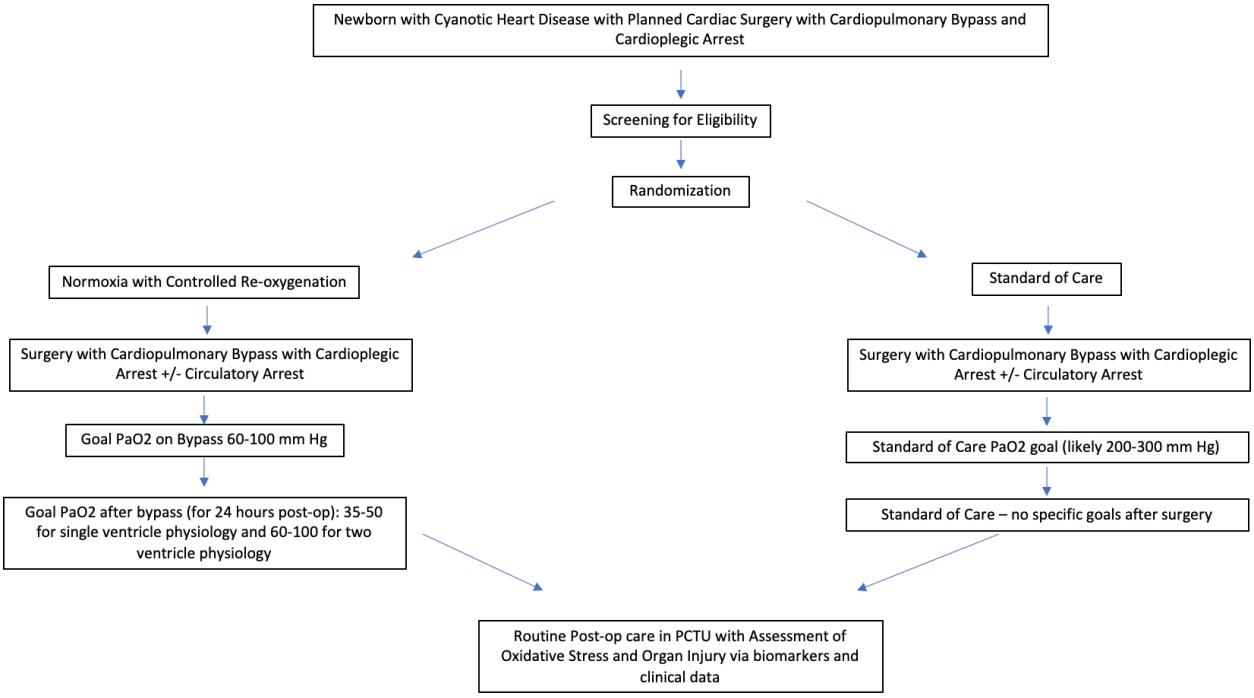
After cardiac surgery, the following tests and evaluation will be performed:

1. At 2, 6, and 24 hours after surgery (time marked as decannulation from cardiopulmonary bypass): biomarkers for oxidative stress, inflammation, vascular injury, neurologic, myocardial, and renal injury will be drawn.
2. At 24, 48, and 72 hours after surgery: renal function and liver function panels will be obtained
3. A head ultrasound will be performed at 24 and 72 hours after surgery
4. EEG monitoring may be connected to each infant after arrival to the ICU as part of the standard of care.

6. Adverse Event Assessment

Assessment of Serious Adverse Events will be performed 1month post surgery. A chart review will be performed by the study team. If a chart review can't be accomplished, the study team will contact the patient via phone call for an assessment. See Serious Adverse Event section of the protocol for specific definitions.

Figure 2: Study protocol flow chart



Outcomes and Analysis:

Analysis will be performed on the basis of the intent-to-treat principle. Baseline characteristics will be compared between the treatment groups using standard univariate tests.

Specific Aim 1: The primary outcome for this aim will be systemic oxidative stress based on thiobarbituric acid reactive substances (TBARS), a measurement of lipid peroxidation, assessed at three separate time points in the first 24 hours after surgery. An analysis of co-variance will be used incorporating all available data to model the outcome with an adjustment for pre-operative baseline (although we anticipate no differences in baseline values by treatment arm due to randomization). The model will compare the mean values (and 95% confidence intervals) between the two groups at each time-point. In this model, we will assume missing data are missing at random, i.e., missing values may depend on observed but not unobserved data. Analysis will be performed on the basis of the intent-to-treat principle and will include an interim analysis after 24 patients are enrolled. In secondary analyses, we will use propensity scores to

account for any characteristics that were not balanced by treatment arm after randomization. We plan to analyze other biomarkers of oxidative stress and organ injury using analogous analysis of variance models.

Specific Aim 2: To evaluate safety, we will assess the rate of observed adverse events between groups using Chi-square test or Fisher's exact test as appropriate. Safety will also closely be monitored on a routine basis by the Data Safety and Monitoring Board (DSMB). .

Specific Aim 3: To investigate optimal endpoints in the study of the clinical impact of normoxia. We will assess clinical endpoints that may be reflective of a reduction in oxidative stress, including post-operative length of stay, days alive and out of the intensive care unit (ICU) at 30 days after surgery, a composite outcome of major adverse events as described above, and a global rank score. For all endpoints, data on distribution within our study population and between the two groups will be calculated to understand effect sizes and aid in planning subsequent studies.

1. Post-operative length of stay: Calculated as number of days in the hospital after surgery. May be an attractive outcome as it can incorporate many of the morbidities that would be influenced by strategies to reduce oxidative stress as most would lead to prolonged length of stay. The disadvantages are that it does not incorporate mortality, and may be influenced by other center care processes and practices.

2. Days alive and out of the ICU or hospital at 30 days after surgery: This endpoint has been used to measure outcomes in other adult trials and a limited number of pediatric trials. It can incorporate both mortality and various morbidities that may be influenced by strategies to reduce oxidative stress as most significant complications or end-organ injury can extend stay in the ICU or hospital. As a continuous variable, it is also statistically expedient allowing for smaller sample sizes to detect significant differences in treatment groups.

3. Composite outcome: A composite endpoint can combine multiple relevant morbidities or adverse events such as those described above. Disadvantages are that all are inherently assumed to be of equal severity.

4. Global rank score: The rank score has the advantage of allowing for the combination of various endpoints into a single quantifiable endpoint like a composite outcome, however also allows weighting of the severity of the component endpoints, as opposed to a traditional composite endpoint where all endpoints are treated equally. It has been utilized increasingly in other trial settings. In this study, scores will be calculated based on a pre-specified ranking of outcomes: mortality, cardiac arrest, ECMO, seizures, and dialysis. We will also explore the inclusion of other clinical endpoints in both the global rank score and composite outcome, as well as different ranking strategies.

Other secondary exploratory outcomes of general morbidity and end-organ dysfunction will be measured and compared between the two treatment groups. Please see Table 3 for a description of the biomarkers and other tests that will be collected or performed.

<u>Other Secondary Exploratory Outcomes:</u>

1. Neurologic:
    a. Post-operative intracranial hemorrhage and stroke as measured by Head ultrasound
        i. Alternatively, the diagnosis can be made via Head CT or MRI if clinically indicated by the treatment team
    b. Biomarkers for neurologic injury (GFAP, Tau)
    c. Incidence of seizures (clinical or sub-clinical by EEG)
2. Respiratory:
    a. Mechanical ventilation time (total during hospital stay)
    b. Time from surgery to first extubation attempt
    c. The T3 Etiometry system will be used to calculate indices of lung compliance (Tidal volumes, mean airway pressures) on admission, 2, 6, and 24 hours after surgery (or until extubation)
3. Heart/Circulatory system:
    a. Incidence of arrhythmias requiring medical therapy

b. VIS score on admission, 2, 6, and 24 hours post-operatively

c. Measurement of arteriovenous saturation difference:

i. Regional venous saturation measurements (NIRS), which are routinely collected in all patients (measurements will be recorded at 2, 6 and 24 hours after surgery)

ii. Pulse oximetry measurements and arterial oxygen saturation (via arterial blood gas) at 2, 6, and 24 hours after surgery

d. Lactic acid production (highest and lowest value in 12-hour increments in the first 48 hours after surgery). Arterial blood gases are routinely obtained in the post-operative period

e. Need for stress dose hydrocortisone in the post-operative setting

f. Biomarkers: troponin release, vascular injury (ANGPT2) and inflammatory balance (IL-6, IL-10)

4. Kidneys:

a. % above dry-weight daily for the first 5 post-operative days (Dry weight will be defined as dosing weight which will likely be the birth weight of the infant)

b. Days to dry weight

c. Serum creatinine levels (comparison of pre-op, daily trends in the first 3 days including identification of peak level)

d. Biomarkers: NGAL (urine)

5. Oxidative stress:

a. Whole blood redox potential

b. Biomarkers (protein carbonyls, total antioxidant capacity (TAC), and 8-isoprostanes)

6. GI:

a. Liver function tests and intestinal fatty acid binding protein (I-FABP (pre-operatively, 24, 48, and 72 hours post-operatively)

b. Time to goal feeds

Table 3: Schedule of Evaluations

* SOC: test performed as part of Standard of Care. R: Test performed as part of research

** Note: blood volume is extra amount that is needed for research purposes only.

*** Can be run with already collected samples per SOC

Test	Pre-op (within 24hrs prior to surgery)	Baseline (in OR prior to bypass)	End Rewarming	2 hour post	6 hour post	24 hour post	Morning of POD 1	Morning of POD 2	Morning of POD3	General Post-Op for 48 hours	1 month Post-op
Blood **	**0 mL**	**2.75 mL**	**2.25 mL**	**2.75 mL**	**2.75 mL**	**2.75 mL**	**0 mL**	**0 mL**	**0 mL**		
Renal Function Panel	**X SOC**						**X SOC**	**X SOC**	**X SOC**		
Liver Function Panel ***	**X R**						**X R**	**X R**	**X R**		
TBARS		**X R**	**X R**	**X R**	**X R**	**X R**					
Protein Carbonyl		**X R**	**X R**	**X R**	**X R**	**X R**					
8-isoprostane		**X R**	**X R**	**X R**	**X R**	**X R**					
Total Antioxidant Capacity		**X R**	**X R**	**X R**	**X R**	**X R**					
Redox Potential		**X R**	**X R**	**X R**	**X R**	**X R**					
i-FABP		**X R**		**X R**	**X R**	**X R**					
Troponin		**X R**		**X R**	**X R**	**X R**					
GFAP		**X R**	**X R**			**X R**					
Tau		**X R**		**X R**	**X R**	**X R**					
ANGPT-2		**X R**		**X R**	**X R**	**X R**					
IL-6		**X R**		**X R**	**X R**	**X R**					
Il-10		**X R**		**X R**	**X R**	**X R**					
Urine											
Urine: NGAL		**X R**		**X R**							
Neuroimaging and Testing											
Head Ultrasound	**X R**						**X R**		**X R**		
EEG										**X SOC**	
Miscellaneous (Collected or Measured as SOC or Based on Clinical Care Team											
NIRS (Cerebral and Renal)				**X SOC**	**X SOC**	**X SOC**				**X SOC**	
Arterial Blood Gas with Lactate				**X SOC**	**X SOC**	**X SOC**				**X SOC**	
Adverse Events											
Assessment of Serious Adverse Events											**X R**

Description of Risks/Benefits and Monitoring Strategy

Benefits:

The possible benefits of the study will be that the patient's family and care team will receive extra information regarding their cardiovascular and health status. The evaluation of organ function and injury after surgery may provide valuable information to the family and the clinical team that would not otherwise be available, and this may lead to early intervention if abnormalities are detected. Although an individual patient may not directly benefit from the study participation, the results will help to further the understanding of oxidative stress and organ injury after cardiopulmonary bypass and will hopefully allow for further future studies that enroll a larger number of patients.

If parents decline to participate, their child's medical care will not be affected in any way. Furthermore, if they agree to participate, they are free to withdraw at any time.

Risks:

Definition of Serious Adverse Event:

An extensive review of the literature and survey results from other centers with cardiopulmonary bypass strategies similar to what is proposed indicate that there is a low probability of additional risk to the patients. In this trial, due to the risks of cardiac surgery itself, assessment of relationship of the intervention to any adverse event is challenging.

Consistent with standard clinical trials practice, a Serious Adverse Event is defined generally as any event that:

(a) Is fatal; or

(b) Is life-threatening (the subject was, in the view of the Principal Investigator, in immediate danger of death from the event as it occurred); or

(c) Is severely or permanently disabling; or

(d) Necessitates significant intervention, such as major surgery, to prevent permanent impairment of a body function or permanent damage to a body structure; or

(e) Necessitates or prolongs hospital admission; or

(f) The Principal Investigator, medical monitor, or DSMB considers a serious adverse event.

Because many normal post-operative outcomes in this study would meet these criteria, only the following six events have been identified as specific Serious Adverse Events for the purposes of this study:

(a) Death

(b) Cardiac arrest requiring CPR and medications

(c) Cardiopulmonary insufficiency requiring ECMO

(d) Seizures

(e) Need for dialysis

In addition, any other event that in the opinion of the study investigator is considered to be a Serious Adverse Event may be classified as such and will then adhere to the rules for expedited reported. If a clinical event or finding does not meet the definition of "serious" per above, these non- serious events will be classified and will be reported as a complication rather than as a serious adverse event.

Classification of Serious Adverse Event

All Serious Adverse Events will be classified based on whether the event was expected, the relationship to the intervention, and the clinical outcome. Specific definitions and classifications are described below.

All Serious Adverse Events will be evaluated as to whether their occurrence was expected or whether it was not expected to occur.

A). "Expected": An event is considered expected if it is known to be associated with the underlying cardiovascular anomaly or with congenital heart surgery, and is mentioned in the protocol, informed consent, or other study documents. An event may be expected despite the study subject's clinical state immediately prior to the event. For this protocol, expected events include:

(a) Sudden cardiac death

(b) Arrhythmias

(c) Tamponade

(d) Postoperative bleeding

(e) Postoperative open chest

(f) Postoperative chest re-exploration

(g) Postoperative electrolyte abnormalities

(h) Postoperative fever

(i) Postoperative renal dysfunction

(j) Postoperative liver dysfunction

(k) Shunt thrombosis

(l) Aortic coarctation with or without associated ventricular dysfunction

(m) Ventricular dysfunction not associated with coarctation

(n) Pericardial effusion

(o) Wound infection

(p) Reintubation after extubation following surgery

(q) Hypoxia

(r) Respiratory distress

(s) Pleural effusions

(t) Seizures

(u) Renal dysfunction associated with ventricular dysfunction

(v) Liver dysfunction associated with ventricular dysfunction

B). "Unexpected". An event is considered unexpected if there are no prior data linking this event with either the condition or intervention under study.

In terms of relation to the intervention, the Serious Adverse Event will be classified as:

a) definitely related to the intervention

b) probably related to the intervention

c) possibly related to the intervention

d) unlikely to be related to the intervention

e) definitely not related to the intervention

The clinical outcome of the Serious Adverse Event will be characterized as follows:

(a) Death

(b) Recovered: the patient returned to baseline status

(c) Symptoms continue

Data Safety Monitoring Board (DSMB)

A Data and Safety Monitoring Board will be established. The DSMB will meet 4 times a year to review study conduct, adverse events, and any interim data.

After each DSMB meeting, a Summary Report of Adverse Events will be prepared within 30 days and will be distributed to the Principal Investigator with instructions that the Principal Investigator forward the Summary Report to the IRB. The Summary Report will contain the following information:

- A statement that a DSMB review of outcome data, adverse events, and information relating to study performance took place on a given date
- A statement as to whether or not the frequency of adverse events exceeded what was expected and indicated in the informed consent
- A statement that a review of recent literature relevant to the research took place
- The DSMB's recommendation with respect to progress or need for modification of the protocol or informed consent. If the DSMB recommends changes to the protocols or informed consent, the rationale for such changes and any relevant data will be provided

Reporting of Adverse Events/Serious Adverse Events

Reports of Adverse Events/Serious Adverse Events will be submitted to the local Institutional Review Board (IRB) per institutional guidelines. The PI will report any serious adverse event to the DSMB Chair as soon as possible and no later than 7 calendar days after the event.

All events/complications and all events reported as serious adverse events will be tabulated and reviewed by the DSMB at their regularly-scheduled meetings, at least 4 times per year.

Data Collection Procedures for Adverse Events/Serious Adverse Events

1. The five events specified as Serious Adverse Events are to be reported in an expedited fashion. Any other event that in the opinion of the study investigator is considered to meet the definition of a Serious Adverse Event should also be reported in an expedited fashion.

Significant peri-operative and other in-hospital adverse events will be collected and be reported as "complications" versus adverse events.

2. Any medical condition or abnormal laboratory value present at enrollment that remains unchanged or improves will not be reported as a serious adverse event or complication. However, worsening of a medical condition that was present at enrollment will be considered a new event and reported according to the plan outlined above.

3. The onset of an event/complication will be recorded according to the date of the first presenting symptom.

4. Serious adverse events will be reviewed by the Principal Investigator and the DSMB.

References:

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APPENDIX A: Acronym List

ANGPT2: Angiopoietin2

CPB: Cardio-Pulmonary Bypass

CT: Computed Tomography

ECMO: Extracorporeal membrane oxygenation

EEG: Electroencephalogram

EF: Ejection Fraction

FiO2: Fraction of Inspired Oxygen

GFAP: Glial fibrillary acidic protein

GI: Gastro-Intestinal

ICU: Intensive Care Unit

I-FABP: Intestinal fatty-acid binding protein

IL: Interleukin

IQR: Interval Quartile Range

MRI: Magnetic Resonance Imaging

NIRS: Near-infrared spectroscopy

PaO2: Partial Pressure of Oxygen

PCTU: Pediatric Cardiothoracic Intensive Care

PFT: Pulmonary Function Test

POD: Post-Operative Day

TBARS: Thiobarbituric Acid Reactive Substance

VIS: Vasoactive-Inotropic Score

VSD: Ventricular Septal Defect