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Delayed Rewarming for Neuroprotection in Infants Following Cardiopulmonary Bypass Surgery: A Safety Study

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INTRODUCTION

Therapeutic hypothermia (TH) is a medical treatment used for neuroprotection in certain clinical scenarios where there is a substantial risk of brain injury. TH is thought to improve neurological outcome by decreasing cerebral metabolism, reducing blood-brain barrier disruption and attenuating free-radical mediated and excitatory neurotransmitter-mediated pathology. TH during cardiac surgery has been in routine use since the 1950's and is also considered a recommended treatment for adults who have experienced cardiac arrest according to both the American Heart Association (AHA) and the International Liaison Committee on Resuscitation (ILCOR) guidelines. Amongst adult cardiac arrest patients, there has been recent controversy regarding whether hypothermia (cooling to 33°) or the avoidance of **hyper**thermia (aggressive maintenance of 36°) provides superior neuroprotection. A recent publication in the New England Journal of Medicine of 939 adults with out-of-hospital cardiac arrest did not show a difference in survival or neurological outcome between these two management strategies[1]. While the depth and duration of TH remains controversial in adult patients, use of therapeutic hypothermia in newborn infants with moderate to severe encephalopathy and signs of intrapartum asphyxia is considered the current standard of care, with multiple pilot studies demonstrating safety[2,3] and multiple additional randomized control trials demonstrating efficacy[4-6]. Eleven randomized trials comprising more than 1500 infants were evaluated in a Cochrane review on this subject and use of TH resulted in a "statistically significant and clinically important reduction in the combined outcome of mortality and major neurodevelopmental disability to 18 months of age."[7] The proposed research project will focus on the use of TH and the management of rewarming after TH in infants with congenital heart disease (CHD) following congenital heart surgery. Despite significant advances in surgical approaches to CHD, developmental outcomes for the 40,000 affected infants born each year remain disproportionately poor[8,9]. Following congenital heart surgery, infants demonstrate lower than expected intelligence quotients, hypotonia, microcephaly, language delay and poor functional outcomes with less independence in daily activities compared to typical peers[10-12]. These suboptimal developmental outcomes are the rationale for reassessing the current standard of care.

BACKGROUND AND HYPOTHESIS

At Maine Medical Center, TH is routinely employed for 24 hours in the care of adult patients with cardiac arrest and for 72 hours in newborns with signs of intrapartum asphyxia. Both these patient populations are slowly "rewarmed" (brought back to normothermia) over a period of 12 hours in a carefully controlled manner. This strategy for slow rewarming was developed due to the re-emergence of seizures, a clinical sign of brain irritability and injury, in infants more rapidly rewarmed over 6 hours[13-15]. Patients who undergo cardiac surgery (adults, children and infants) however, are typically rapidly "rewarmed" to normothermia at a rate of 1°C each 3-5 minutes, due to concerns surrounding the risks associated with prolonged time on the cardiopulmonary bypass machine, such as higher rates of thrombus formation, embolic events or even hemorrhages. For an infant cooled to 18°C during surgery, it may take only 54 minutes to rewarm to 36°C.

There is emerging evidence that rapid temperature changes of this magnitude may be deleterious to the very neurons that TH is being employed to protect. Animal data suggest that such rapid rates of rewarming may diminish or even negate the neuroprotective effect of hypothermia used during surgery[16,17]. In human trials, adults and children have demonstrated significant cognitive deterioration if rapidly rewarmed to normothermia on cardiopulmonary bypass[18,19]. These studies have used a combination of serum biomarkers (s100b and Neuron Specific Enolase) that are indicative of brain injury and performance on cognitive testing as outcome variables.

S100b is a glial-derived calcium binding protein that is a biomarker for brain injury in multiple populations, including adult patients with traumatic brain injury and stroke and neonates with hypoxic ischemic brain injury[20]. S100b has also been studied in adults and children following cardiopulmonary bypass[21-23] and found to be elevated with correlations established between poorer neurological outcomes and elevated s100b levels. Neuron specific enolase (NSE)[24] is a glycolytic enzyme concentrated in the cytoplasm of neurons and another biomarker of cerebral injury that can be easily measured like s100b from a serum sample. Both s100b and NSE were assessed in term encephalopathic newborns treated with TH and were found to be associated with developmental outcome at 15 months with higher levels of either biomarker reflecting poorer developmental outcome[25]. S100b as a biomarker has also been assessed in children with congenital heart disease in the post-operative setting. In a 2013 study, 80 children ages 6-15 years, who received surgery for correction of a common congenital heart defect, were treated with either the standard of care (rapid rewarming to normothermia in the operating room on bypass) versus a delayed rewarming technique (which stopped rewarming on bypass at 33°C and permitted passive rewarming to normothermia over the next several hours)[19]. The children in the delayed rewarming group had lower s100b levels in the postoperative setting compared to the standard of care group. In addition, the delayed rewarming group showed no post-operative deterioration in neuropsychological function compared to the standard of care group, which displayed post-operative deterioration across all domains including information, comprehension, arithmetic, digit span and verbal IQ. These findings in older children are compelling and suggest that similar investigation is needed in infants.

In this proposed pilot study, the safety of delayed rewarming will be assessed in infants under the age of 12 months who require cardiopulmonary bypass surgery for repair of congenital heart defects. **It is proposed that the delayed rewarming technique will be safe and that a trend toward lower serum biomarker levels will be seen in the experimental group compared to the standard of care group.** Successful completion of this pilot safety study will lay the foundation for a future adequately powered efficacy study to determine if the delayed rewarming technique leads to improved developmental outcomes.

Specific Aim 1: Evaluate safety of delayed rewarming in infants following congenital heart disease surgery through intensive care unit level monitoring of parameters including hemodynamic status, coagulopathy, infection, hyperthermia and subcutaneous fat necrosis. In this pilot study, infants under the age of 12 months with congenital heart disease requiring surgical palliation with cardiopulmonary bypass will be randomly assigned to the standard of care versus experimental group. The experimental treatment is "delayed rewarming" and will be defined as the cessation of rewarming on cardiopulmonary bypass at 35°C. There will be 12 hours of controlled rewarming to 36.5°C via the use of a programmable body temperature regulating blanket that is FDA approved for this use. Safety will be determined by continuous close monitoring in the post-operative setting in the Pediatric Intensive Care Unit for adverse effects on hemodynamic status (bradycardia, junctional ectopic tachycardia, hypotension and need for additional medication to support heart rate or blood pressure), for coagulopathy (lab evidence of disseminated intravascular coagulation, chest tube output and need for transfusion of blood products), infection (such as pneumonia), effects on other end organs (monitored via lab testing for kidney function daily), frequency of infants with **hyper**thermia and lastly close inspection of the skin to insure there is no evidence for subcutaneous fat necrosis. My hypothesis is that the "delayed rewarming" group will not demonstrate a higher frequency or severity of adverse events compared to the standard of care group.

Specific Aim 2: Compare early and late biomarkers for adverse developmental outcomes between standard of care and "delayed rewarming" groups including serum biomarkers of brain injury, electroencephalogram (EEG) and developmental outcomes.

2.1 Compare levels of serum biomarkers of brain injury in the acute post-operative setting including serum s100b and NSE (neuron specific enolase). Levels will be assessed in both groups in the pre-operative setting for baseline and

serially in the post-operative setting for 72 hours. It is hypothesized that infants in the "delayed rewarming" group will show a trend toward lower levels of biomarkers of brain injury.

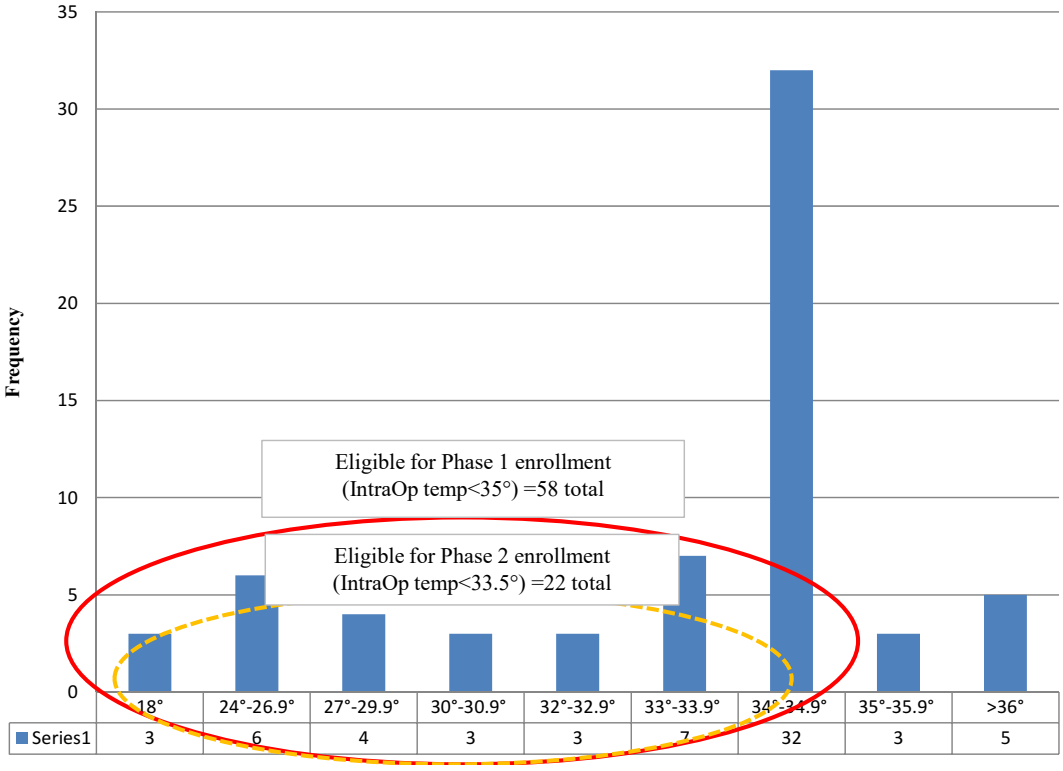
2.2 Record EEG and monitor seizure frequency in infants for 48 hours following CHD surgery. Seizures, if they occur, will be treated in both groups and the duration quantified in seconds. EEG is needed primarily to rule out potential confounding effects of seizures on later developmental outcome. It is hypothesized that there will be infrequent seizures and no differences in seizure frequency or duration between groups.

2.3 Compare developmental outcomes using Bayley Scales of Infant Development (Bayley 3). Bayley testing will be performed on infants at either 6 or 12 months of age depending on the infant's age at the time of surgery. It is hypothesized that infants in the "delayed rewarming" group will show a trend toward improved Bayley scores compared to the standard of care group.

Preliminary data:

A retrospective review of cases of congenital heart disease requiring surgical palliation was performed for cases from January 2011 to May 2014. The database was provided by Cheryl Jones to Dr. Alexa Craig for the purposes of this analysis. This database is kept for quality work in Dr. Reed Quinn's office. Please see the attached letter of support for this project from Dr. Quinn. In this review, there were 191 surgeries performed on infants under the age of 12 months by Dr. Quinn in the 41 month interval (average 4.6 surgeries per month). Some infants required multiple surgeries and therefore the total number of surgeries (191) reflects 119 individual infants. The proposed research project includes only infants undergoing cardiopulmonary bypass (CPB) for the first time. In this sample, there were 88 CBP surgeries. Ten infants had surgery requiring CPB on more than one occasion and thus eliminating repeat exposures to cardiopulmonary bypass, 76 of the 88 total surgeries would have met inclusion criteria for enrollment into this research project (1.9 infants per month or 22 per year). Anticipating a 2 year period of recruitment, there should be approximately 44 eligible infants and we will seek to enroll 30.

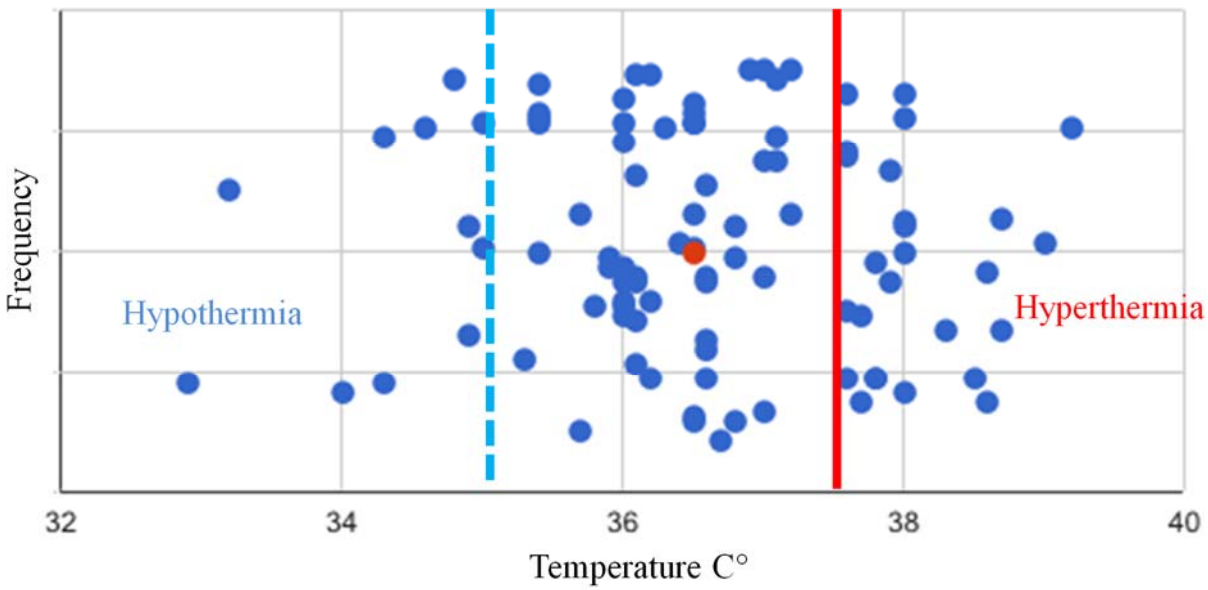
Figure 1: Lowest IntraOperative Temperature 1/2011-5/2014



Retrospective analysis of the lowest intraoperative temperature was also performed and data could be recovered for 66 of the 76 total eligible infants. The lowest intraoperative temperature was 18° for 3 infants, the highest intraoperative temperature was 36° for 5 infants and the mean intraoperative temperature was 30.1°. The most frequently occurring intraoperative temperature was in the range of 34-34.9° with 32 cases (48%) over the 41 month time period (see Figure 1). Applying inclusion criteria for the proposed study, 58/66 (88%) would be eligible for Phase 1 of the project with a lowest intraoperative temperature of less than 35° (red circle). Extrapolating this to the whole number of 76 infants, there would have likely been 67 infants who would meet inclusion criteria (1.6 per month x 24 months recruitment period=39 possible recruits). When considering the inclusion criteria for Phase 2, 22/66 (33%) infants from the retrospective cohort had a lowest intraoperative temperature of less than 33.5° (yellow dashed circle). Extrapolating this to the whole number of 76 infants, there would have likely been 25 infants who met inclusion criteria (1.0 per month x 24 months recruitment period=24 possible recruits).

In a different retrospective review of congenital heart surgery cases under the age of 6 months (2008-2013), there is evidence of significant variability in post-operative temperatures on arrival to the Pediatric Intensive Care Unit (PICU) from the Operating Room (OR). The mean arrival temperature was 36.6°C, with a range of 32.9° to 39.2°. Eleven infants were significantly hypothermic upon arrival to the PICU ranging from 32.9° to 35° (see Figure 2) and all but 2 survived without evidence of any immediate hemodynamic compromise or coagulopathy. One infant died 2 weeks following surgery from necrotizing enterocolitis and the other 3 weeks following surgery from severe brain injury. Although this is a small number, there is no evidence for a higher mortality rate with infants who arrived to the PICU hypothermic as proposed in this research project.

Figure 2: Temperature in C°-arrival to Pediatric ICU



In the same review from 2008-2013, fourteen infants arrived to the PICU with temperatures above 38°C, with 2 of those over 39°C and as such would be considered **hyper**thermic (see Figure 2). Of the 14 **hyper**thermic PICU arrivals, 1 died 2 weeks following surgery from heart failure. This preliminary data demonstrates a significant need for better post-operative temperature management as it is well established in the traumatic brain injury and stroke literature that **hyper**thermia is associated with worse outcomes. This preliminary data also suggests that by rewarming to 36-36.5°C according to the current institutional protocol on cardiopulmonary bypass, at least one third of infants then develop "overshoot" of their temperature beyond 37°C. This post-operative **hyper**thermia may also be corrected with use of the delayed rewarming strategy proposed in this project and as such is a potentially modifiable source of neurological injury.

MATERIALS AND METHODS

Subjects and study design: 30 infants under the age of 12 months with congenital heart disease requiring cardiopulmonary bypass surgery will be recruited for this study. The experimental treatment is "delayed rewarming" and will be defined as the cessation of rewarming on cardiopulmonary bypass at 35°C (see Figure 3). During both phases, there will be 12 hours of controlled rewarming to 36.5°C and the temperature will be maintained at 36.5 C for the 12 hours after rewarming . All types of congenital heart disease requiring cardiopulmonary bypass and intraoperative hypothermia will be considered for enrollment including (Tetralogy of Fallot (TOF), Arterioventricualr canal (AV canal), Hypoplastic left or right heart (HLHS), Transposition of the Great Arteries (TGA), Truncus arteriosus (TA) and Total Anomalous Pulmonary Venous Return (TAPVR). Exclusion criteria include a pre-existing coagulopathy or cardiac lesions not requiring hypothermia and bypass and these include Atrial Septal defect (ASD), Ventricular Septal Defect (VSD), Patent Ductus Arterious (PDA) and Coaractation of the Aorta (Coarc). Exclusion criteria also includes the inability to wean from bypass following surgery This is a randomized pilot study primarily assessing the safety of the delayed rewarming technique and secondarily assessing levels of serum biomarkers for neuronal injury at the 4 intervals shown below (see Figure 3).

Figure 3a: Research design for pilot

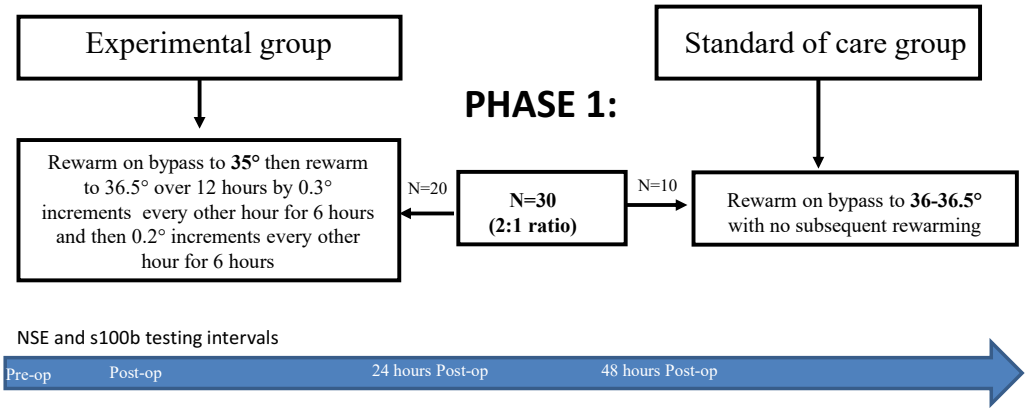
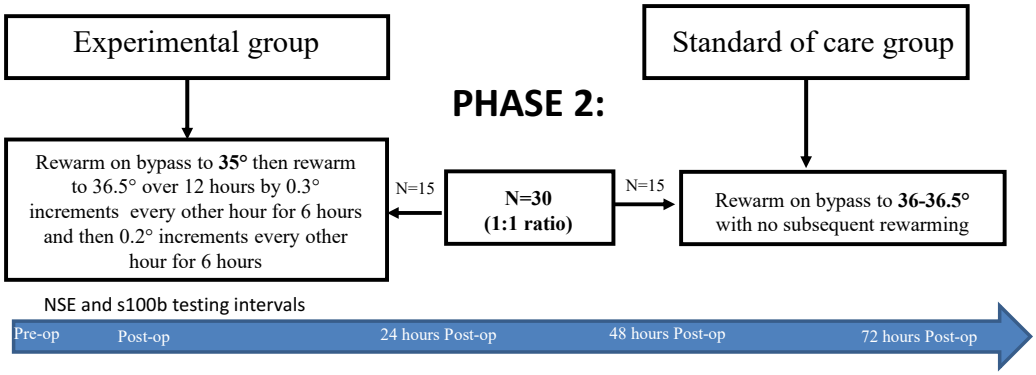


Figure 3b: Research design for KL2 project



Randomization: We will randomize in a one to one fashion. A random number generator will be used with those numbers being translated into a list of sequentially numbered opaque envelopes that will be opened by the principal investigator (PI) in order at the time of randomization following the informed consent process.

Recruitment: Dr. Adrian Moran and Dr. Shari Wellen are pediatric cardiologists and follow expectant mothers weekly or biweekly throughout a pregnancy known to be complicated by a diagnosis of congenital

heart disease. Dr. Moran and Dr. Wellen will refer appropriate patients to Dr. Craig (PI) for consideration of enrollment. The PI will meet with the pregnant mother and father in the outpatient setting several weeks after the diagnosis has been made, giving the family time to come to terms with this new diagnosis. At the first meeting with the PI, the family will be informed about the research study and given informational handouts. Another meeting will occur prior to the admission during which the congenital heart surgery will be performed. At this second meeting, informed consent documentation will be reviewed and signatures obtained from parents willing to participate. For infants who need congenital heart surgery within the first few days of life or for those in whom a fetal diagnosis was not made, the PI will obtain informed consent from parents in the hospital prior to surgery. The envelopes for randomization will be opened at the time consent is obtained to insure that there is adequate time to arrange for the type of post-operative care the infant will receive (intervention versus standard of care).

Standard of care group: For infants assigned to the standard of care group, the congenital heart surgery will be performed according to usual practice with the degree of intra-operative hypothermia determined by the cardiothoracic surgeon and his team based on the anticipated complexity of the case. Following completion of the surgical procedure, the infant will be rapidly rewarmed on cardiopulmonary bypass at a rate of 1°C each 3-5 minutes. The infant will achieve normothermia of 36-37°C and be transported to the Pediatric Intensive Care Unit (PICU) for routine post-operative monitoring. Standard of care procedure in the post-operative setting is clearly defined below on Figure 4.

Figure 4: Standard of Care Temperature Management *after* Bypass

LOCATION	PERSON RESPONSIBLE	RECORDING SITE	GOAL TEMP	FACTORS
Operating Room	Anesthesiologist	Nasopharyngeal	36-37°C	1. Bair Hugger: consider 38°C setting rather than 43°C setting 2. Blood products do not necessarily have to be warmed prior to administration-titrate to infant temperature 3. iv Tylenol administered in the OR (10 mg/kg)
Transport to PICU	Anesthesiologist	Nasopharyngeal	36-37°C	1. Do **NOT** automatically place warm blankets on infant 2. OR room temperature should be >73°F (23°C)to reduce radiation and convection losses 3. Anesthesiologist to continue Nasopharyngeal temperature monitoring until the infant is departing for PICU
PICU	CT surgeon and/or PICU attending	Esophageal	36-37°C	1. Monitor temperature continuously-record hourly 2. Room temperature >73°F (23°C) 3. Tylenol orders placed for ongoing therapy (+6 hours from dose given in OR)
If infant develops **HYPOthermia (CORE temp <36°C)**				1. Apply Bair hugger and set to 38°C 2. Close doors to room 3. Increase ambient room temperature >75°F 4. Call CT surgeon/PICU Attending for temperature <35.5°C
If infant develops **HYPERthermia (CORE temp >37.0°C)**				1. Remove blankets on infant 2. Check room temperature-decrease if necessary 3. If temperature climbs to >37.0°C, apply ice packs to axilla and groin, recheck temp Q 15 min, remove ice when temperature <37°C again 4. Call CT surgeon/PICU attending for temperature > 37.5°C

Routine monitoring includes careful observations of the following parameters:

1. Hemodynamic status- The components of hemodynamic status that are continuously monitored in the postoperative setting include heart rate, rhythm and blood pressure. In the post-operative setting, telemetry (continuous heart rate monitoring) is standard on all congenital heart patients and all patients have arterial lines placed for continuous blood pressure monitoring. These are direct measures of hemodynamic status and are continuously uploaded to the electronic medical record on a minute to

minute basis. Nurses at the bedside will record these measurements at a minimum of every 60 minutes for the first 48 hours after surgery. If hemodynamic status is compromised, kidney function may be altered and therefore blood urea nitrogen (BUN) and creatinine levels will be assessed daily for the first 48 hours after surgery (may be more frequently at the discretion of the team managing the patient).

2. Respiratory status- Most infants return to the PICU intubated and their respiratory rate and oxygen saturation levels are also continuously monitored. Ventilator support is titrated by the medical team and the respiratory therapist according to results from blood gases which are obtained at regular intervals depending on the acuity of the patient. Chest x-rays are obtained daily to monitor for pneumonia or the development of other fluid collections in the chest (i.e. chylous effusion).
3. Coagulopathy-After congenital heart surgery, some infants have difficulty with coagulation (may occur in part as a result of the anticoagulation medications that are used during bypass and after surgery) and are subsequently at risk for bleeding or clot formation. Disseminated Intravascular Coagulopathy (DIC) is routinely screened for daily with laboratory studies including fibrinogen, INR, platelet count, PT/PTT and D-Dimer. Infants may be treated with Platelets or Fresh Frozen Plasma or Cryoprecipitate if coagulopathy develops. Chest tube output is also monitored in cc/kg
4. Infection- The nurse and medical team will monitor for signs of infection including fever and appearance of the surgical wound. Central line related infections and urinary catheter related infections are also monitored for and these lines are removed as soon as is feasible to decrease this risk.
5. **Hyper**thermia-Hyperthermia is undesirable in a post-operative congenital heart patient. Nurses routinely follow temperatures at the same intervals detailed above for the other vital signs and will treat infants who develop hyperthermia with anti-fever medications.

Three deviations from routine care will occur in the standard of care group.

1. Electroencephalogram (EEG) will be performed in the immediate 48 hours following surgery to monitor for the occurrence of seizures. EEG leads are applied to the scalp of the infant and are non-invasive. The infant will have residual effects of anesthesia from surgery and is unlikely to experience any discomfort from the application of the electrodes on the scalp. The EEG will be remotely assessed by the PI at a minimum of 3 times per 24-hour period. Current American Clinical Neurophysiology Society guidelines recommend a minimum of 2 daily EEG assessments[26]. If seizures occur, the EEG recording will be marked for seizure onset and seizure end so that the total duration of seizures in seconds may be calculated. If seizures are detected in either standard of care or delayed rewarming groups, they will be treated according to established guidelines which include administration of a standard loading dose of phenobarbital (20 mg/kg) followed by a second loading dose of phenobarbital in the event of persistent seizure activity. In the unlikely scenario that seizures are still present, Fosphenytoin would be administered in a 20 mg/kg loading dose. The type of medications administered including dose and timing of administration will be recorded in the electronic medical record and compared between groups.
2. Residual blood that would otherwise be wasted from a clinically obtained sample will be collected and used for testing for biomarkers of neuronal injury (s100b and NSE). These samples will be obtained at the following 5 intervals; the pre-operative setting for baseline, post-operatively after bypass, at post-op hour 24, at post-op hour 48 and at post-op hour 72. Dr. Anne Breggia, Research Program Director of the BioBank and Research Laboratory Services (RLS) at Maine Medical Center Research Institute has kindly agreed to assist with technical support for the processing of the s100b and NSE testing. The s100b testing is a serum ELISA test run on a kit provided by Millipore™. This test requires 100µL of serum per sample to be tested. This ELISA may be run on a sample that has been previously frozen. The NSE test is also run on serum and is an ELISA test requiring 100µL of serum that may also be used on a frozen stored specimen. The company providing the NSE sample kit is R&D Systems. The stored samples will then be run in batches once the full collection period is complete. The collection of the samples and the handling of samples will be facilitated by the PI and Debbie Cushing, research

coordinator participating in the project (please see the attached letter of support from Kathryn Cope, VP Neuroscience Service Line who is funding the research coordinator position).

3. Bayley Infant Scales of Development (Bayley 3) will be performed between age 9 months and 2 years. The testing will be performed by a physical therapist who assesses fine and gross motor indices and by a Master's level psychologist who assess both the cognitive and language development components. Severe developmental disability will be defined as a Bayley Mental Development Index score that is 2 standard deviations below the mean score (eg. below 70)[5]. Moderate developmental disability will be defined as a Bayley Mental Development Index score that is 1 to 2 standard deviations below the mean score (eg. below 70-84)[5]. The parents of research subjects will have the opportunity to meet with a member of the developmental assessment team to review results of the testing.

Experimental group: For infants assigned to the experimental group, the congenital heart surgery will be performed according to usual practice as detailed in the standard of care group section above, with depth of hypothermia dictated by the complexity of the congenital heart lesion to be repaired. Upon completion of the surgical procedure, partial rewarming will occur on cardiopulmonary bypass, but will stop when the infant reaches 35°C.

The infant will be transported from the operating room to the PICU at 35°C and placed on a servo-controlled blanket with an esophageal temperature probe placed. Through use of the servo-controlled blanket, the rate of rewarming will be carefully regulated with increases in temperature of 0.3°C every 2 hours for 6 and then of 0.2°C every 2 hours for 6 hours to the goal temperature of 36.5°C (see Appendix 1 for temperature management worksheet phase 1). The intensity and frequency of post-operative monitoring will be exactly the same in the experimental group as in standard of care group, including post-operative EEG, serum biomarker testing and neurodevelopmental follow up. The following parameters will be observed in the experimental group and compared with the standard of care group.

1. Hemodynamic status-Infants who are hypothermic typically have lower resting heart rates than those who are normothermic (80-100 beats per minute versus 120-140 beats per minute respectively). In the post-operative congenital heart infant, bradycardia (slow heart rate) could conceivably have an adverse impact on the hemodynamic status of the infant and if untreated this could also impact renal function. Hemodynamic status will be continuously monitored in the same manner as detailed in the standard of care section. Intravenous medications to support cardiac function and blood pressure (which these infants are typically already treated with) will be up-titrated if necessary. The need for permanent pacemaker implantation will be documented.

   Normothermic or hyperthermic post-operative congenital heart infants may experience a cardiac arrhythmia following surgery known as junctional ectopic tachycardia (JET). JET is a surgical complication that is concerning due to the fact that the newly repaired heart is beating significantly faster than it is intended to, adding significant strain to the heart muscle and potentially compromising hemodynamic stability. One of the treatments for JET is to reverse **hyper**thermia (this is only an option if the patient has a fever) or potentially even use **hypo**thermia for refractory cases. We will monitor carefully to determine whether or not there is a decreased incidence of JET in the experimental group.
2. Coagulopathy- In adults who undergo therapeutic hypothermia following cardiac arrest, there is evidence of coagulation dysfunction and subsequent risk of bleeding. This tends to not be the case in infants who tolerate therapeutic hypothermia better than adults. Laboratory screening for coagulopathy will occur as described in the standard of care group and if coagulopathy develops, blood products (Platelets or Fresh Frozen Plasma or Cryoprecipitate) will be administered to reverse this. Chest tube output will also be carefully monitored in cc/kg/hour and the number of days a chest tube is in place will also be recorded .
3. Infection- In adults, there is evidence for increased rate of ventilator acquired pneumonia in patients undergoing therapeutic hypothermia after myocardial infarction. Again, this is unusual in infants who

tolerate hypothermia better. The infants in this study will receive daily chest x-rays in the post-operative setting and signs of infection will be carefully monitored for and antibiotics started if necessary.

4. **Hyper**thermia-The infant's temperature will be closely monitored during rewarming and for 48 hours afterward. An esophageal probe must be used during rewarming due to the fact that a skin or rectal recording is less accurate with hypothermic patients.
5. Subcutaneous fat necrosis-In infants who are exposed to a full 72 hours of therapeutic hypothermia, there is a rare complication of breakdown of the fat stores in the skin leading to the formation of fluid filled nodules in the skin that are unsightly but not life threatening. These nodules typically resolve without intervention over the weeks following the hypothermia exposure. Good hemodynamic status is protective against this complication. It is expected that the short duration of rewarming (only 12 hours) will not cause any incidents of subcutaneous fat necrosis.

Anticipated outcomes: The primary outcome variable is safety of the delayed rewarming technique which will be determined by the occurrence of adverse events in the standard of care versus delayed rewarming group. Specifically, clinically significant changes in the hemodynamic status of the infant, coagulopathy, infection or the presence of subcutaneous fat necrosis will be evaluated (see Table 1 for full definitions of all parameters). The secondary outcomes include the comparison of serum biomarkers (s100b and NSE), EEG findings including seizure frequency and developmental outcome assessments in the standard of care versus delayed rewarming group. It is anticipated that the delayed rewarming technique will be safe and that a trend will be seen toward lower level of s100b and NSE in the delayed rewarming groups, although this study is not powered to definitively assess this. Given that this project is considered more than "minimal risk", a data safety monitoring plan has been developed (see attached DSMB plan). The DSMB will determine whether or not an adverse event is clinically significant and whether or not it is attributable to the delayed rewarming intervention. The PI will report any adverse event to the monitoring entity with 24 hours.

In order to carefully define what is considered an adverse event, a retrospective chart review was performed for 15 infants in the past 12 months who survived congenital heart surgery with cardiopulmonary bypass at MMC. Of the 15 infants, the mean age at the time of surgery was 148 days with a range from 4 to 356. Of the 15 infants, 10 were male and 5 female. These infants had a mix of cardiac lesions similar to those who would be enrolled in this study. See Table 1 for a full outline of parameters that will be considered acceptable for infants enrolled in the study, which are based on the retrospective review of 15 survivors. Using these parameters to define what has been previously consistent with "safe", any infant enrolled in the proposed study with results outside these parameters will then be considered as an adverse event and the data safety monitoring board will be informed. If a severe adverse event occurs in either Phase 1 or Phase 2 of the proposed research protocol, no additional infants will be enrolled and all experimental exposures will be suspended until a decision is made by the DSMB.

Table 1: Definitions of Serious, Moderate and Other Adverse Events

Serious Adverse Events	Reported to DSMB within 24 hours of occurrence
Mortality	Death within 48 hour period following surgery
Need for resuscitation	Need for cardiopulmonary resuscitation within 48 hours of surgery
ECMO after surgery	Persistent need for bypass (ECMO) following surgery; **STUDY EXCLUSION CRITERIA**
Return to OR on day of surgery	Unexplained hemorrhage prompting return to the OR for exploratory surgery
Stroke or seizures	Ischemic or hemorrhagic stroke **OR** status epilepticus **OR** clinical or electrographic seizures
Moderate Adverse Events	Reported to DSMB within 48 hours of occurrence
Bradycardia	Clinically significant heart rate <100 bpm for >30 minutes*
Tachycardia	Clinically significant heart rate > 160 bpm for 30 minutes*
Arrhythmia (JET)	Any occurrence of JET(Junctional ectopic tachycardia) **OR** other arrhythmia
Hypotension	Systolic BP< 70 mm Hg **OR** Diastolic BP< 30 mm Hg *
Coagulopathy	INR > 2.2 OR PTT > 74 seconds Thrombocytopenia with platelets < 50,000 Chest tube output more than 5 cc/kg/hr **OR** 40 cc/kg in an 8 hour shift
Leukopenia	White blood cell count < 4,000
Other adverse Events	Reported to DSMB within 7 days
Hypertension	Systolic BP > 100 mmHg **OR** Diastolic BP > 80 mmHg*
Pressor Use	Treatment with more than 2 pressors for more than 7 days
Days of intubation	Intubated for longer than 5 days
Infection	Occurrence of urinary tract infection, pneumonia **OR** other wound infection
Subcutaneous fat necrosis	Any occurence

*resulting in hemodynamic compromise and/or need to administer medications to support heart rate and/or blood pressure

Regarding management of patients with complications from the delayed rewarming intervention, the most likely complication from the delayed rewarming intervention is bradycardia during the 12 hour rewarming period. Given that the post-operative temperature starts at 35°C, the bradycardia is likely to be minor. If bradycardia develops, providers at the bedside will assess the hemodynamic status of the infant. A determination will be made regarding whether or not the bradycardia is resulting in any degree of hemodynamic compromise. Hemodynamic compromise will be defined by bradycardia plus hypotension, poor renal function or oliguria. If there is hemodynamic compromise, then pressors will be titrated to increase the heart rate and blood pressure. These infants are typically already treated with two pressors in the immediate post-operative setting. Rapid rewarming may be considered if increasing pressors does not resolve the issue. In other populations of infants, however, rapid rewarming has been found to increase the severity of adverse events. Specifically, in term newborns with peripheral pulmonary hypertension, rapid rewarming exacerbates their condition by further diverting blood away from the lungs (to the large vascular bed in the skin that was just rapidly rewarmed). For this reason, rapid rewarming would only be used as a second step if pressor titration was not successful.

ANALYSIS

Data will be collected at the bedside and through chart review. A study ID number will be assigned to each infant and study documents will all use this ID number to combine data. Identifiable data will be stored separately. Preoperative (baseline) variables including gender, age in months and type of congenital heart lesion will be collected. A baseline s100b and NSE will also be considered pre-operative variables. Intraoperative variables including aortic cross clamp time, cardiopulmonary bypass time, lowest temperature during surgery, time for rewarming on bypass in all 3 groups and s100b and NSE at the completion of cardiopulmonary bypass will be collected. Postoperative variables include temperature, heart rate, blood

pressure, presence or absence of an arrhythmia such as JET, pressor use, frequency of transfusions, hours of intubation, coagulation dysfunction, frequency and type of infection and presence or absence of subcutaneous fat necrosis for 48 hours after surgery. S100b and NSE will be compared at the 4 post-operative time points as well.

Success of randomization will be assessed by comparing baseline characteristics in the two treatment groups; we acknowledge that randomization may not be entirely effective with such a small sample size and we will examine the data for biases to be considered in interpreting our findings. For this safety study, primary outcomes will be composite outcomes and will include the frequency of serious adverse events as well as the frequency of any adverse events (see Table 1) and will be compared among treatment groups using chi square tests or Fisher's exact tests, as appropriate. The frequency of individual adverse events such as hemodynamic compromise, arrhythmia, coagulopathy, infection, **hyper**thermia and subcutaneous fat necrosis will also be examined. Biomarkers (s100b and NSE) will be examined using a repeated measures analysis of variance, with the time * treatment interaction serving as the hypothesis test.

Additional project support: This clinical project will take place at Maine Medical Center in the Pediatric Intensive Care Unit. I have the support of Dr. Reed Quinn, pediatric cardiothoracic surgeon, Robert Groom, chief perfusionist, Dr. Dan Kovarik, pediatric anesthesiology, Dr. Maribeth Hourihan, pediatric cardiology, Dr. Sandra Bagwell, pediatric intensive care physician and Dr. Anne Breggia, MMCRI. Please see the attached documentations of departmental support. I also have the support of the Pediatric Intensive Care Unit nurses and respiratory therapists who provide post-operative care for these infants. The equipment needed for implementing the delayed rewarming technique was purchased by Maine Medical Center for this research project. The disposable cooling blankets that the infants will be placed upon as well as the disposable temperature probes were purchased from the KL2 grant.

PROTECTION OF HUMAN SUBJECTS

Maine Medical Center (MMC) is committed to protect the rights and welfare of all research subjects. We received accreditation for our human research protection program (AAHRPP) in 2011. All investigators who are engaged in research are required to submit their research to our Institutional Review Board for review prior to conducting their research. We also provide a program for continued oversight and monitoring of all projects. Human Subject Protection and Health Information Privacy and Security training through CITI is a mandatory component of training for all personnel involved in this study as part of the Human Research Protection Program (HRPP) policy at Maine Medical Center. This research proposal, including recruitment materials and the informed consent will be submitted to the IRB for review and approval. This study will be performed in accordance with 45 CFR 46 subpart D, which applies to all research involving human subjects and additional protections for children involved as subjects in research, conducted, supported or otherwise subject to regulation by a federal department or agency.

Human Subjects Involvement, Characteristics and Design: The goal of this research is improve developmental outcomes in infants who have undergone congenital heart surgery. Infants who require surgical correction of congenital heart defects are often treated with hypothermia during the surgery to protect the brain. Following completion of congenital heart surgery, the current standard of care is to rapidly rewarm them on cardiopulmonary bypass to normothermia. This rapid rewarming is thought to have adverse effects on the brain. This project proposes to partially rewarm infants to 35°C on bypass and then complete rewarming over the first 12 hours after surgery in the intensive care unit using an FDA approved machine that can carefully control body temperature changes by small increments. In this project, safety of the

"delayed rewarming" technique will be assessed and we will ultimately aim to assess efficacy in a larger future trial if safety can be shown first.

The study of human subjects is therefore necessary to achieve the goals presented in this application. The proposed clinical research for specific aims 1 and 2 will involve human subjects prospectively recruited from Maine Medical Center located in Portland, Maine. As the only pediatric tertiary care center in the state, the catchment area is large. Included subjects will be less than 12 months of age. Each subject must undergo congenital heart surgery with cardiopulmonary bypass before the age of 12 months. We aim to recruit 30 infants over a 24 month period. It is expected that there will be an equal proportion of male and female subjects. It is expected that the racial and ethnic composition of subjects will reflect the racial homogeneity of the state of Maine.

Infants will be randomized to one of two groups; the standard of care group versus the experimental group. Infants in the experimental group will receive the delayed rewarming therapy, which consists of stopping rewarming on bypass at either 35 °C and gradual rewarming over 12 hours to normothermia through use of a servo-controlled temperature regulating blanket. Adverse events such as effects on hemodynamic status, coagulation status, rate of infection and incidence of subcutaneous fat necrosis will be compared between groups as will serum biomarkers of neuronal injury.

In terms of research subjects, infants with congenital heart disease are considered a vulnerable population and the proposed use of this vulnerable population requires additional justification. Infants with congenital heart disease are not only vulnerable in a research sense, but also in a clinical sense. These infants could not survive without the surgical procedures we have the capacity to perform. Yet, ironically in the face of these tremendous surgical advances, neurodevelopmental outcome remains suboptimal. It is well-established that hypothermia is well tolerated in a different population of infants, namely newborns with suspected hypoxic ischemic brain injury. Multiple safety and efficacy trials have been performed and have shown that not only do these infants tolerate therapeutic hypothermia with exceedingly infrequent complications, but they also demonstrate better developmental outcomes than would otherwise be anticipated based on the clinical history of their births. Infants with congenital heart disease must be studied directly in the clinical setting in order to ascertain whether or not they might benefit in terms of developmental outcomes in the same manner. Before efficacy of the intervention can be assessed, safety must first be demonstrated. Animal models would be inadequate both due to an inability to recreate surgical heart lesions in rodents or other mammals and due to the inability to fully translate animal developmental outcomes into human outcomes.

Sources of Materials: For this protocol, the frequency of adverse events will be compared between the standard of care group and the experimental group across 5 specific areas as follows: hemodynamic status, coagulopathy, infection, hyperthermia and subcutaneous fat necrosis (see next section for full details). The current standard of care for intensive care unit monitoring is adequate to monitor for adverse events in both the standard of care and experimental groups. Adverse events will be clinically apparent and both the event and medical management will be documented in the infant's medical record. Continuous vital signs (temperature, heart rate, blood pressure, respiratory rate and oxygen saturation) will be monitored and recorded by the nurse caring for the patient as well as all usual standard post-operative laboratory and imaging studies. These will include laboratory tests for evidence of renal dysfunction, coagulation studies and chest x-rays monitoring for infection and fluid accumulation. The equipment used to provide delayed rewarming to the 15 infants in the experimental group will be purchased by the research study.

In order to protect the subject information described above, HIPAA standards will be adhered to and data that is kept will be de-identified. Each participant will be assigned an individual participant number and names and medical records will not be used. All paper documentation will be kept in a secure file in a locked area and will ultimately be scanned to pdf and stored on a secure and encrypted server. When

creating the REDCap data base, the subject number will be used as the principal identifier. Dr. Craig and her Research Coordinator, Deb Cushing, RN will be the only persons with access to the key which encodes the subject name with the demographic and other information.

Potential Risks: There are potential risks to the critically ill subjects enrolled in this study, the most significant of which are outlined below:

6. Hemodynamic status-Infants who are hypothermic typically have lower resting heart rates than those who are normothermic (80-100 beats per minute and 120-140 beats per minute respectively). In the post-operative congenital heart infant, bradycardia (slow heart rate) could conceivably have an adverse impact on the hemodynamic status of the infant and if untreated this could also impact kidney function. Hemodynamic status will be monitored and intravenous medications to support cardiac function and blood pressure (which these infants are typically already treated with) will be up-titrated if necessary.
   Normothermic post-operative congenital heart infants may experience a cardiac arrhythmia following surgery known as junctional ectopic tachycardia (JET). JET is a surgical complication that is concerning due to the fact that the newly repaired heart is beating significantly faster than it is intended to, adding significant strain to the heart muscle and potentially compromising hemodynamic stability. One of the treatments for JET is to reverse **hyper**thermia (this is only an option if the patient has a fever) or potentially even use **hypo**thermia for refractory cases. We will monitor carefully to determine whether or not there is a decreased incidence of JET in the delayed rewarming group.
7. Coagulopathy- In adults who undergo therapeutic hypothermia following cardiac arrest, there is evidence of coagulation dysfunction and subsequent risk of bleeding. This tends to not be the case in infants who tolerate therapeutic hypothermia better than adults. Laboratory screening for coagulopathy will occur and if coagulopathy develops, blood products (Platelets or Fresh Frozen Plasma or Cryoprecipitate) will be administered to reverse this.
8. Infection- In adults, there is evidence for increased rate of ventilator acquired pneumonia in patients undergoing therapeutic hypothermia after myocardial infarction. Again, this is unusual in infants who tolerate hypothermia better. The infants in this study will receive daily chest x-rays in the post-operative setting and signs of infection will be carefully monitored for and antibiotics started if necessary.
9. **Hyper**thermia-The infant's temperature will be closely monitored through use of a rectal temperature probe during the delayed rewarming process. A rectal probe must be used due to the fact that a skin recording is less accurate with hypothermic patients.
10. Subcutaneous fat necrosis-In infants who are exposed to a full 72 hours of therapeutic hypothermia, there is a rare complication of breakdown of the fat stores in the skin leading to the formation of fluid filled nodules in the skin that are unsightly but not life threatening. These nodules typically resolve without intervention over the weeks following the hypothermia exposure. Good hemodynamic status is protective against this complication. It is expected that the short duration of rewarming (only 12 hours) will not cause any incidents of subcutaneous fat necrosis.

The alternatives to the proposed delayed rewarming treatment are to continue to offer only the standard of care treatment of rapid rewarming on bypass. This standard of care of rapid rewarming is suspected of having a contribution to the cognitive deficits and neurological dysfunction experienced by patients after congenital heart surgery and cardiopulmonary bypass. Given that it is known that newborn infants with hypoxic ischemic brain injury have essentially no adverse events related to hypothermia, it is reasonable to try this delayed rewarming approach to see if it will be safe in infants following congenital heart surgery. There is a research publication on children ages 6-15 years with congenital heart disease who were treated with delayed rewarming after cardiopulmonary bypass. In this age group, there were no statistically significant differences in adverse events between the delayed rewarming and standard of care groups, but a definite improvement in the cognitive performance of children in the delayed rewarming group. The fact that this delayed rewarming technique has been shown already to be safe and effective in an older population

of children in the post-operative setting with congenital heart disease also makes it reasonable to test this technique in younger children.

The privacy risk to participating subjects is deemed to be minimal and certainly not in excess of that risk incurred with receiving medical care in the first place.

ADEQUACY OF PROTECTION AGAINST RISKS:

Recruitment plan and consent procedure: The pediatric cardiology team and pediatric cardiothoracic surgeon will refer appropriate patients to the principal investigator (PI) for consideration of enrollment. The PI will meet with families in the outpatient setting to obtain consent prior to the admission during which the congenital heart surgery will be performed. For infants born who need congenital heart surgery within the first few days of life, the PI will obtain consent from parents in the hospital prior to surgery. The envelopes for randomization will be opened at the time consent is obtained to insure that there is adequate time to arrange for the type of post-operative care the infant will receive (intervention versus standard of care).

Before infants are accepted into the study, the parent or guardian must:

- give oral and written consent
- be aware of alternative treatments, and that alternative clinical care is available;
- be aware of the right to withdraw

In a private location, following discussions about informed consent with the parent/guardian, we will ask the parent or guardian to sign an authorization to participate in research and permission to use or release protected health information (informed consent). Parents of potential participants who do not comprehend or speak the English language will be offered a personal translator through Maine Medical Center's (MMC's) language bank.

The inclusion criteria specify that all participants will be both male and female infants under the age of 12 months with congenital heart disease requiring cardiopulmonary bypass for surgical correction. All ethnic groups will be eligible. Subjects will be recruited only from Maine Medical Center.

Exclusion criteria specify that infants with known coagulopathy or those not requiring cardiopulmonary bypass or hypothermia during surgery will not be enrolled.

Protection Against Risks: The Institutional Review Board (IRB) at Maine Medical Center will oversee the safety of subjects in this research study. Initial approval is required before the study is initiated and during its conduct.

Risk for adverse effects of the delayed rewarming strategy will be monitored by a drug safety and monitoring plan (see below). Adverse events will be reported to the members of the monitoring board and the interpretation of these events being related to the delayed rewarming intervention will be made by members of the board.

Potential Benefits of the Proposed Research: There are significant potential benefits to the subjects in this research. Current practice for post-operative pediatric congenital heart patients at Maine Medical Center includes rapid rewarming, which is thought to contribute to cognitive dysfunction and neurological abnormalities. The delayed rewarming technique proposes to ameliorate this effect although this study in not powered to demonstrate this. In addition, infants may not experience an arrhythmia (junctional ectopic tachycardia) as a result of better temperature regulation that prevents **hyper**thermia and may have better cardiac performance as a result of this. If infants have seizures, these will be better recognized and treated

with 48 hours of continuous EEG in the post-operative setting. If development is adversely affected, early developmental assessment could lead to earlier referral for therapy which may help outcome.

Importance of the Knowledge to be Gained: Cognitive and developmental outcomes for infants following congenital heart surgery are suboptimal in many cases. The proposed delayed rewarming strategy uses an FDA approved device for an approved indication in a new population of patients. If the delayed rewarming strategy is safe and well-tolerated, subsequent studies will be designed to assess efficacy. We believe that this research is greater than minimal risk but presenting the prospect of direct benefit to the individual child and to the population of infants with congenital heart defects (according to 45 CFR 46.405). It is also conceivable that the delayed rewarming strategy could be implemented in children and adults in similar post-operative settings.

Data Safety and Monitoring Plan: Three independent medical monitors have been appointed for this study to review clinical events in real time to allow timely, unbiased decision-making regarding study risk. The data safety monitoring board (DSMB) will consist of Dr. Maribeth Hourihan, attending pediatric cardiologist, Dr. Thomas Reynolds, attending pediatric neurologist and Dr. Kristine Pleacher, attending pediatric intensive care physician. None of these three physicians were directly involved in the design or implementation of this research study and are therefore as unbiased as one can be in the assessment of safety. They have been selected to serve on the DSMB due to their combined experience and ability to critically assess clinical scenarios. The PI will report any adverse event to the monitoring entity with 24 hours and any other care provider to the infant will have the ability to report adverse events as well. The Data Safety Monitoring plan will follow the procedures and policies outlined by the Human Research Protection Plan at Maine Medical Center (MMC). This plan requires that the study be reviewed by the Maine Medical Center Institutional Review Board (IRB), and upon approval the conduct of the study will adhere to the Good Clinical Practice Standard Operating Procedures for Good Clinical Practice in the Conduct of Clinical Research at MMC. A Study Coordinator from the MMC Clinical Trials unit will be monitoring on a continuous basis the collection of study data for this research project. The Study Coordinator will maintain all regulatory files and ensure that all data collection is monitored against source documentation. The Study Coordinator will also ensure proper monitoring for adverse events as well as protocol deviations, following guidelines contained in the Clinical Practice Standard Operating Procedures for Good Clinical Practice in the Conduct of Clinical Research at MMC.

Definitions of Adverse Events and reporting to DSMB

Table 1: Definitions of Serious, Moderate and Other Adverse Events

Serious Adverse Events	Reported to DSMB within 24 hours of occurrence
Mortality	Death within 48 hour period following surgery
Need for resuscitation	Need for cardiopulmonary resuscitation within 48 hours of surgery
ECMO after surgery	Persistent need for bypass (ECMO) following surgery; **STUDY EXCLUSION CRITERIA**
Return to OR on day of surgery	Unexplained hemorrhage prompting return to the OR for exploratory surgery
Stroke or seizures	Ischemic or hemorrhagic stroke **OR** status epilepticus **OR** clinical or electrographic seizures
Moderate Adverse Events	Reported to DSMB within 48 hours of occurrence
Bradycardia	Clinically significant heart rate <100 bpm for >30 minutes*
Tachycardia	Clinically significant heart rate > 160 bpm for 30 minutes*
Arrhythmia (JET)	Any occurrence of JET(Junctional ectopic tachycardia) **OR** other arrhythmia
Hypotension	Systolic BP< 70 mm Hg **OR** Diastolic BP< 30 mm Hg *
Coagulopathy	INR > 2.2 OR PTT > 74 seconds Thrombocytopenia with platelets < 50,000 Chest tube output more than 5 cc/kg/hr **OR** 40 cc/kg in an 8 hour shift
Leukopenia	White blood cell count < 4,000
Other adverse Events	Reported to DSMB within 7 days
Hypertension	Systolic BP > 100 mmHg **OR** Diastolic BP > 80 mmHg*
Pressor Use	Treatment with more than 2 pressors for more than 7 days
Days of intubation	Intubated for longer than 5 days
Infection	Occurrence of urinary tract infection, pneumonia **OR** other wound infection
Subcutaneous fat necrosis	Any occurence

*resulting in hemodynamic compromise and/or need to administer medications to support heart rate and/or blood pressure

Stopping Rules: DSMB has the responsibility of pausing or stopping the trial in the case of 1) any serious adverse event probably or definitely related to study intervention OR 2) two or more unexpected or unanticipated adverse events of same or similar type

Adequacy of sample: We aim to enroll 30 infants and randomize 15 to the standard of care and 15 to the experimental group. With 15 infants in each group, we have determined that we will not have the necessary power to permit definitive analysis of the results, but will use these preliminary data to establish whether or not the treatment is safe and well-tolerated for the design of future, larger adequately powered trials.

Inclusion of Women and Minorities: The recruitment for this study requires that infants have a diagnosis of congenital heart disease requiring surgical palliation. The incidence of congenital heart disease is estimated at 1 percent of births in the United States or 40,000 infants per year. In Maine, there are nearly 13,000 births annually and the possibility of 130 new infants each year with congenital heart disease. Surgery with cardiopulmonary bypass is performed only on the most severely affected and estimated at approximately 60 per year under the age of 12 months. As the incidence of this disease is random, so too will be the distribution of affected boys versus girls. The parents of every child with severe congenital heart disease will be approached for participation and this is feasible since our institution is the only hospital in the state where these surgeries can be performed. Maine is a homogenous state with respect to minorities, but we do however have increasing immigrant and refugee populations which will be approached equally. The PI will work to ensure that there are no disparities in the recruitment of boys versus girls or of Caucasian versus minority infants.

Inclusion of Children: Congenital heart disease occurs in children and often requires surgical palliation within the first 12 months of life. These infants who undergo surgery for congenital heart disease are at risk for cognitive and neurological dysfunction due in part to the side effects of the very procedures they require in order to sustain life. It is necessary to prospectively enroll infants with congenital heart disease who have undergone corrective surgery to determine whether or not delayed rewarming is safe and well-tolerated to permit future study of the efficacy of this technique. All subjects enrolled in this study will be infants under the age of 12 months.

The PI has experience in clinical research with neonates and extensive clinical training providing her with the expertise necessary to enroll and safely monitor the neurological care of these infants.

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