Anesthesia and the Developing Brain: a Comparison of Two Anesthetic Techniques

Study Protocol and Statistical Analysis Plan

NCT03882788

March 14, 2012

Project Title: The effect of anesthetic agents on neurodevelopmental outcome in neonatal congenital heart disease patients
COMIRB #06-0483
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Study Objectives:

Primary objective:
To evaluate whether the type of anesthesia, narcotic-based versus inhalation-based (volatile) anesthesia, administered during cardiopulmonary bypass (CPB) surgery contributes to the wide variance in neurologic recovery and developmental outcomes after surgery in infants with congenital heart disease.

Secondary objectives:
1. To evaluate whether levels of metabolites in blood and urine using magnetic resonance spectroscopy preoperatively, intraoperatively and 2-5 days postoperatively can predict postoperative neurologic recovery and outcome.
2. To examine whether postoperative neurodevelopmental status changes can be predicted by EEG (seizure activity) within the first two days after surgery.

Background and Significance:
Thirty thousand infants are born each year in the United States with congenital heart disease. Half of these infants require surgical intervention within the first year of life. More importantly 8-25% of these children develop neurologic sequelae, which can have a long-term detrimental effect on IQ and behavior (Bellinger 1991; duPlessis 1997; Hovels-Gurich 2001; Karl 2004). The question is whether there were predisposing neurologic factors preoperatively or were there intraoperative factors that led to the postoperative neurologic outcomes.

Limperopoulos and colleagues have provided the strongest evidence of preoperative neurologic problems with both a formal neurobehavioral assessment and neurologic exam (Limperopoulos et al. 1997). Using the Einstein Neonatal Neurobehavioral Assessment Scale (ENNAS), they were able to identify neurobehavioral and neurologic deficits in more than half of full term infants with cardiac defects, cyanotic and non-cyanotic, prior to surgical intervention. Most studies conclude that preoperative neurobehavioral evaluations and long-term developmental follow-up of infants undergoing cardiac surgery are very important. However, many institutions do not offer routine neurologic and developmental follow-up of these at risk infants.

When drawing conclusions about the utility of differing strategies of CPB management and effects on neurologic outcome, one must consider the preoperative neurologic state. Some studies find no difference in outcome and others report significant deficits occurring more often

with total circulatory arrest than low flow CPB (Limperopoulos 1999; Miller 1996; Brunberg 1974; Bellinger 1991; Hesz 1988). These studies were isolated to a single condition, transposition of the great arteries, and none of these studies examined the preoperative baseline neurologic or developmental status of the infants in order to be able to assess changes postoperatively. Furthermore, the question remains does the type of anesthetic predict later neurologic outcome?

Recent evidence from animal research in rodents supports that anesthetics may cause neurodegeneration in the developing brain (Jevtovic-Tedorovic 2003). This has been further supported by research in our own laboratory. Neurodegeneration was most evident in rat pups, 7 days in age and exposed to anesthesia for 5 hours. Clearly, age and duration of anesthesia had an impact on anesthesia induced neurodegeneration (Faberowski 2004). The pathognomic anesthetic for a prolonged anesthetic exposure in infants is that used for CPB. The studies from Bellinger and others were during a period where high dose fentanyl was the primary anesthetic. Currently, volatile anesthetics seem to be the more commonly used anesthetic because of the work of Kurth and others. Kurth et al (2001, 2002) demonstrated improved histiologic outcome in piglets who received a volatile anesthetic as opposed to a fentanyl anesthetic.

Perioperative brain imaging studies have also been useful in documenting CNS injury such as hypoxic-ischemic encephalopathy and focal cortical infarctions (Gaynor 2003; Tavani 2004; Mahle 2003). Recently, serial proton magnetic resonance spectroscopy of the brain was used to evaluate children under two different intraoperative strategies: low flow versus normal flow CPB and low versus high hematocrit during CPB (Ashwal 2004). In this series of eleven infants, long and short echo time single voxel magnetic resonance spectroscopy in occipital gray matter and neurologic assessment were obtained preoperatively and at two and five days postoperatively. Changes in brain N-acetyl-aspartate, and increases in myoinositol and glutamate/glutamine after surgery were sensitive in detecting postoperative neurologic changes; however, these changes could not be correlated to a difference in bypass strategy because of the limited number of children studied. Additionally, according to Newburger et al. (1993) and Rappaport (1998), postoperative seizure activity within 48 hours after CPB was most predictive of poor neurologic outcome at one year of age.
The blood brain barrier in children less than one year of age is disrupted by CPB. We wish to study these changes in N-acetyl-aspartate, and increases in myoinositol and glutamate/glutamine via the use of blood and urine samples rather than cerebral spinal fluid (Cavaglia M 2004). We wish to avoid lumbar puncture in a heparinized child and also avoid the transport of an unstable child to the MRI scanner, thus limiting our study to blood and urine specimens only. We would like to confirm our findings via blood and urine with perioperative EEG monitoring for 48 hrs seizure activity and Bayley III testing at 18-24 months of age in the two anesthesia (narcotic-based vs. volatile) groups.

Patient Accrual/Selection of Study Population:
Neonates (children 30 days in age or less) diagnosed with congenital heart disease will be evaluated and potentially recruited from the NICU or CICU. It is anticipated that 1 to 2 patients per week, approximately 50-100 patients will be eligible for the study in one year. Assuming at least 90% survival to discharge and 30% refusal, there will be 35-60 infants who could be recruited per year. We wish to recruit 200 patients over 5 years. Assuming a 70-75% return rate, approximately 30-50 infants are expected to return for their 18 month follow-up.

Inclusion/Exclusion Criteria:

Inclusion Criteria:
- All neonates less than 44 weeks post conceptional age age and greater than 32 weeks post conceptional age admitted to The Children's Hospital for treatment of cyanotic heart disease requiring surgical intervention.
- Admitting diagnosis of cyanotic heart disease.

Exclusion Criteria:
- Infants >44 weeks post conceptional age or <32 weeks post conceptional age on admission.
- Infants with the diagnosis of acyanotic heart disease
- Infants with documented central nervous system malformations.
- Infants requiring unexpected postoperative ECMO support will be excluded.

Consent and HIPAA authorization:
All subjects will be consented prior to participation in this study and prior to randomization. Parents/guardians will be approached by one of the study anesthesiologists after the determination for surgical intervention has been made, usually the day prior to surgery. The study anesthesiologists are all COMIRB certified and experienced in obtaining consent for research studies. The study will be explained to the parents/guardians in a quiet area away from other patients to allow time for questions. Parents will be asked to explain the study in their own words to assess understanding of the study. A HIPAA authorization will be obtained at the same time as consent. Parents/guardians will be given copies of both the consent and HIPAA authorization for their records.

Subject Randomization/Determination of anesthetic maintenance:
All infants enrolled in the study will receive a preoperative assessment by one of the cardiac anesthesiologists. Since all of these infants will have a peripheral intravenous line preoperatively, the induction will be standardized to sevoflurane up to 8%, 2 mcg/kg of fentanyl and 1 mg/kg of rocuronium. Induction is the period in which the airway is secured. This usually takes 5-10 minutes in an otherwise normal infant.

The anesthetic maintenance will be determined using a computer-generated randomization table and assigning each patient to one of the two anesthetic regimens (see Table 1). For instance, subject #1 is enrolled in the study and consent obtained. The randomization table says that subject #1 is assigned to receive a volatile anesthetic, isoflurane. That subject's maintenance will

be with a volatile anesthetic, isoflurane. Isoflurane was chosen as the maintenance anesthetic because it is the only anesthetic vaporizer currently available for our CPB machines.

Table1. Anesthetic maintenance assignment

Random # assignment	Narcotic-based	Volatile
Subject 1		X
Subject 2		X
Subject 3	X	
Subject 4		
Subject 5		X
Subject 6	X	
Subject 7	X	

Anesthetic Technique:
Volatile anesthetic:
In volatile anesthetic technique, maintenance of anesthesia will be standardized to the volatile anesthetic isoflurane. Isoflurane will be used for the study since this is what is presently available on the CPB machines. Anesthesia at 1.0 MAC indicates that at this concentration 50% of the patients will not move when surgically stimulated. We commonly use about 1.2-1.4 MAC in neonates, since the MAC value in infants is higher than that of children and adults. Isoflurane will be delivered at 1.5-2.0%% as required for anesthetic management. These concentrations of isoflurane represent 1.0 to 1.2 MAC of anesthesia. Rocuronium or pancuronium will be used for muscle relaxation. Narcotic, fentanyl will be administered at no greater than 2 mcg/kg/hr. However, the primary anesthetic during CPB will be isoflurane with no narcotic administered during CPB.

Narcotic-based anesthetic:
In narcotic based anesthetic technique, no volatile anesthetics will be used past induction. Maintenance of anesthesia will be with fentanyl 5 mcg/kg/hr not to exceed 10 mcg/kg/hr. The anesthetic may be supplemented with midazolam 0.05 mg/kg/hr but not to exceed 1.0 mg/kg/hr. Narcotic-based anesthetic will be used by the cardiac anesthesia team and the CPB technician throughout the operative case. 5 mcg/kg/hr of fentanyl is felt to represent 0.6 MAC of anesthesia. Total narcotic based anesthetic is also a standard technique in managing neonates undergoing CPB. However, the primary anesthetic during CPB will be fentanyl with no isoflurane administered during CPB

Postoperative Sedative and Analgesic Care:
As per our institutional standard of care, postoperative sedation will consist of fentanyl infusions of 2-10 mcg/kg/hr for the first 48 hours postoperatively.

Data collection:
Detailed chart review and data collection will be performed to obtain data for multivariate analyses including 1) socio-demographic data, 2) presence of metabolic acidosis*, 3) intubation, 4) history of palliative intervention procedure-prostaglandins, atrial septostomy, 5) age at surgery, 6) length of circulatory arrest and duration of CPB, 7) complications of surgery, and 8) head ultrasound or MRI studies if obtained for clinical indications.

* Presence of a metabolic acidosis and increased lactate as result of poor systemic oxygenation can occur in this population secondary to a restrictive ASD and inadequate mixing. The oxygenation provided to the lower body may be initially insufficient in patients with cyanotic heart disease and thus produce metabolic acidosis.

Determination of CNS injury, recovery, and neurodevelopmental outcome.

Table 2: Summary and Timeline of Procedures Performed

Times of testing	NMRS-blood*	NMRS-urine*	EEG	Bayley III Exam
Prior to surgery	X	X	X	
During anesthesia and prior to CPB	X	X	X	
During CPB	X	X	X	
Immediately after CPB	X	X	X	
6 h	X	X	X	
12 h	X	X	X	
24 h	X	X	X	
48 h	X	X	X	
72 h	X	X		
18 – 24 m				X

CPB= CPB, h= hours after CPB, m= months after surgery/CPB, NMRS= Nuclear magnetic resonance spin spectroscopy

* Blood and urine samples will not be required after 120 hours postoperatively. If the arterial line \is removed before 72 hours, this is the endpoint of the study; all samples will be from an arterial line which is a standard of care for anesthesia and critical care monitoring.

NMR spectroscopy of blood and urine samples
Blood samples:
Blood samples will be obtained from a peripheral arterial catheter routinely placed as per protocol for procedures requiring CPB. Blood will be obtained at 9 time points: Prior to surgery, during anesthesia and prior to bypass, during CPB, immediately after bypass, and 6,12, 24, 48 and 72 hours after bypass. Heparin-preserved whole blood (0.5 mL) will be used for NMR analysis. All samples will be placed on ice, then frozen and stored at -78 degree until extraction. Prior to NMR analysis, 0.5 mL of blood will be extracted using a dual methanol/ chloroform extraction developed in our laboratory (Serkova et al., 2005). This allows for protein precipitation and separation of water-soluble and lipid metabolites. Blood will be carefully vortexed and processed with 1 mL of cold chloroform/methanol (1:1, vol/vol). After centrifugation, the supernatants will be collected and the pellets will be re-suspended with 0.5 mL of chloroform/methanol, centrifuged, and the supernatants will be collected. The supernatant will be washed with 0.5 mL ice-cold water. The water phase will be removed and added to the pellet. One mL of water will be added and the pellet will be centrifuged and the supernatant will be lyophilized overnight (water-soluble extracts). Afterwards the water-soluble part will be dissolved in 0.5 mL D_2O and analyzed by proton NMR. The lipids in the organic phase will be

evaporated to dryness under a stream of nitrogen at 50°C. The lipid extracts will be dissolved in 0.6 mL of deuterated chloroform/ methanol (2:1, vol/vol) for ^{1}H-NMR analysis.

Urine collection:
Urine (3 mL) will be collected from a foley catheter routinely placed for surgical procedures requiring CPB at 10 time points: Prior to surgery, during anesthesia and prior to bypass, during CPB, immediately after bypass, and 6,12, 24, 48 and 120 hours after bypass.

Urine and blood samples will be centrifuged to remove possible proteins if present. The samples will be placed on ice, then frozen and stored at -78 degree until extraction. Prior to NMR analysis, the pH will be adjusted to 5.7 using K_2HPO_4/ KH_2PO_4-buffer. The samples will be lyophilized overnight, and re-dissolved in 0.5 mL of D2O for NMR.

Proton quantitative NMR on blood and urine extracts. All water-soluble and lipid extracts will be analyzed using a 500 MHz high-resolution Bruker DRX system. An inverse TXI 5-mm probehead will be used for all experiments. In order to suppress water residue in extracts, a standard Bruker water presaturation sequence will be used ("zgpr") (operating frequency for proton channel: 500.24 MHz; power level pl1=3 dB; power level for water suppression pl9=55 dB; power angle p1=7.5 µsec (90° pulse); power angle for water suppression p12=60 µs; water suppression at O1=4.76 ppm; relaxation delay d1=12.85 sec (5*T1); delay for power switching d12=20 µs; short delay d13=3 µs; spectral width sw=12ppm; total number of scans ns=40). An external standard substance, trimethylsilyl propionic-2, 2, 3, 3,-d_4 acid (TMSP, 20 and 50 mM in D_2O) will be added into a thin glass capillary. The final TMSP concentration (0.5 mmol/L and 1.2 mmol/L) in the capillary will be calculated prior to NMR experiments on study extracts using a standard amino acid solution. The TMSP capillary will be placed into the NMR tube during the experiment (0.5 mmol/L for water-soluble extracts and 1.2 mmol/L for lipid extracts) and served as an external standard which allowed for absolute metabolite quantification in each study extract. ^{1}H chemical shifts will be referred to TMSP signal at 0 ppm. After performing Fourier transformation and making phase and baseline corrections, each ^{1}H peak will be integrated using 1D WINNMR program (Bruker Biospin Inc., Fremont, CA). The absolute concentrations of single metabolites will be then referred to the TMSP integral and calculated according to the equation (1):

$$Cx = \frac{Ix : Nx \times C}{I : 9} \times V : M \quad (1)$$

where Cx = metabolite concentration
Ix = integral of metabolite ^{1}H peak
Nx = number of protons in metabolite ^{1}H peak (from CH, CH_2, CH_3, etc.)
C = TMSP concentration
I = integral of TMSP ^{1}H peak at 0 ppm (:9 since TMSP has 9 protons)
V = volume of the extract
M = volume of blood sample (0.5 mL)

Data interpretation for NMR ("metabolomics"). Urine and blood ^{1}H-NMR spectra will be statistically analyzed by two-phase principle component analysis in order to determine (i) the group clustering (PCA score) and (ii) the marker identification (PCA plot) for volatile anesthetic

group versus the intravenous anesthetic group. Once distinguished by PCA, the changes in absolute concentrations of metabolic markers will be analyzed by un-paired Student t-test.

Neurologic exams and neurobehavioral testing:
At 18-24 months after surgery, a clinical neurologic examination will again be performed by the study neonatologist to determine the presence of abnormal and/or asymmetric muscle tone and persistent or abnormal primitive reflexes. Head circumference and other growth parameters will also be measured. Neurocognitive and motor status will be assessed by a neonatologist using the Bayley Scales of Infant Development- Third Edition (Bayley III), which is the most frequently used standardized assessment of developmental milestone achievement in infants and young children. The Bayley III measures cognitive and motor development resulting in both an index score, comparable to an IQ score, and an age equivalent. The Behavior Rating Scale of the Bayley III combines observation and parent report and is divided into factors measuring attention/arousal, orientation/engagement, emotional regulation and motor quality.

Risks:
There are no additional risks to participating in the study. Both narcotic-based and volatile anesthetics are standard practice at The Children's Hospital.

Benefits:
There is no direct benefit to the patient or the patient's family. The results of this research may help physicians evaluate the efficacy of different anesthetics by better defining the biologic potential for long-term neurologic and neurobehavioral outcome. It may also lead to new approaches to the study and use of neuroprotective agents and better selection of infants who might benefit from early intervention services after discharge, as well as provide a means of more accurately predicting neuropathology, recovery and long term neurodevelopmental outcome in infants with congenital heart disease.

Subject payment:
Families will receive [redacted] to compensate for travel time at the 18-month follow up visit.

Costs to the subject:
There will be no costs incurred upon the subject or family. MRI spectroscopy of blood and urine, EEG studies and Bayley III evaluations will be covered by the research protocol.

Data analysis and monitoring:
The dependent variable or primary outcome of concern is the child's neurodevelopmental status at 18-24 months post surgery for congenital heart disease (CHD). The data used to index neurodevelopmental status include the socioeconomic status, preoperative status- intubation, prostaglandin infusion, atrial septostomy, preoperative diagnosis of cyanotic heart disease with or without a ventricular septal defect, presence of a non-restrictive versus restrictive atrial septal defect, neurological exam, tests of developmental achievement and MRI spectroscopy data. MRI spectroscopy data include: Peak area metabolite levels for N-acetylaspartate, creatinine, choline, and the lactate doublet. Peak area metabolite levels will be calculated as a ratio of the above variables.

The independent variable of concern is the anesthetic, categorized specifically by narcotic-based or volatile anesthetic. Our estimates of sample size requirements assume a conservative 20% (Bellinger 1991; duPlessis 1997; Hovels-Gurich 2001; Karl 2004) incidence in neurodevelopment disabilities, which indicates that we may assess 200 children before reaching the desired proportion of poor outcome cases. However, if the effect is strong, or if the incidence of developmental disability is higher than expected, we may demonstrate at least trends toward differences in outcome groups by the end of two years. Subjects lost to follow up or withdrawn from the study for any reason will be reported and analyzed in a sub group analysis.

A designated statistician will provide data analysis at the end of the study.

Data Safety and Monitoring Plan:

Hard copies of all data collection sheets will be kept in the study coordinator's locked file cabinet in a locked office. Data will be entered in an Excel spreadsheet for later analysis. All investigators have password protected computers with Windows XP operating systems as required for TCH security systems standards. The computers are backed up to the TCH network on a nightly basis so that data will be protected and recoverable in case of a catastrophic computer failure. The subjects will only be identified on the sheet by a study number. The key linking the study number to the subject will be held only by the investigators on password protected computers in a secure office.

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