Title:	**EEG to monitor propofol anesthetic depth in infants and toddlers**
Short Title	EEG propofol depth young children
Study Drug	Propofol
Sponsor:	The Children's Hospital of Philadelphia
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ABBREVIATIONS AND DEFINITIONS OF TERMS

AE	Adverse events
EEG	Electroencephalography
Ce	Effect site concentration
SEF	Spectral Edge Frequency
IV	Intravenous
Laryngoscopy	Using a laryngoscope to visualize the vocal cords.
Intubation	Placement of an endotracheal tube after laryngoscopy
Infant	28 days to 12 months of age
Toddler	12 -24 months of age
Child	2 yrs to 12 yrs of age
LMA	Laryngeal Mask Airway

ABSTRACT

Context: (Background)
Sevoflurane inhalation and propofol intravenous anesthesia are the mainstays of delivering general anesthesia in children. Propofol anesthesia in children is gaining popularity due to fewer respiratory complications, less post-operative nausea vomiting and emergence delirium, compared to sevoflurane. However, unlike sevoflurane, the pharmacodynamics of propofol is less studied in infants and toddlers, particularly the biomarker for propofol brain effect site concentration (Ce), indicative of anesthetic depth. The lack of a real-time biomarker often results in over- or under-dosing of propofol in clinical practice. The goal of this study is to utilize electroencephalography (EEG) as the biomarker of propofol effect site concentration and clinical anesthetic depth, thereby improving the safety and efficacy of propofol anesthesia in this population.

Objectives: (primary and important secondary objectives)
Primary: In infants and toddlers receiving propofol anesthesia, EEG will be recorded while the patient undergoes three stimuli used to assess anesthetic depth (placement of oral pacifier, electrical stimulation, and laryngoscopy). The EEG index (spectral edge frequency-SEF) where 50% of patients do not respond to each of the three stimuli will be determined as the biomarker of propofol clinical anesthetic depth.
Secondary: In the same cohort, the regression between EEG SEF and plasma propofol levels will be determined to assess relationship between EEG SEF and propofol Ce.

Study Design:
Sequential allocation prospective trial.

Setting/Participants:
The study will be performed at CHOP. Up to 150 patients who are undergoing procedures under general anesthesia will be enrolled to produce 120 evaluable patients with 60 in each of two age groups (3-12mo and 13-24mo).

Study Interventions and Measures:
For each patient, up to three stimuli will be done in sequence (placement of oral pacifier, electrical stimulation, and laryngoscopy—a clinically required procedure). Propofol dosing will be adjusted to achieve a target EEG SEF for each stimulus. EEG SEF is used as a biomarker of anesthetic depth. The response to the stimulus and EEG SEF will be recorded, along with obtaining a 1ml blood sample. Obtaining the blood sample may not always coincide with the stimulus and up to three blood samples may be drawn per subject. Between different stimuli on the same patient, adjustments to propofol dosing may be

required to achieve a different SEF target. After each study patient, the response to the stimulus will be used to adjust the EEG SEF target for the next patient.
At the end of the study, the median EEG SEFs (EEG SEF_{50}) for each of the three stimuli will be determined. The propofol Ce that corresponds to each EEG SEF will be determined from a regression curve of SEF *vs* propofol plasma concentration obtained from 1ml of patient blood and measured in a pharmacodynamics laboratory. The study will only be performed by the investigators listed in the study.

PROTOCOL SYNOPSIS

Study Title	EEG to assess propofol anesthetic depth in infants and toddlers
Funder	Departmental funds
Study Rationale	Sevoflurane inhalation and propofol intravenous anesthesia are the mainstays of delivering general anesthesia in children. Propofol anesthesia in infants and toddlers is gaining interest due to fewer respiratory complications, less post-operative nausea vomiting and emergence delirium, compared to sevoflurane anesthesia. However, the pharmacodynamics of propofol has not been well studied in infants and toddlers, and there is no readily available method to monitor the depth of propofol anesthesia, which often results in over- or under-dosing of propofol. Anesthetic dosing and the resultant brain effect-site concentration (Ce) depend on the desired clinical endpoint during a procedure. In clinical practice, the three main endpoints of anesthesia depth are *unconsciousness*, no *response to surgical incision*, and no *response to laryngoscopy*. Electroencephalography (EEG) can be used to monitor anesthetic depth during propofol anesthesia in children and adults, as EEG parameters (spectral edge frequency-SEF) decrease predictably with increasing propofol Ce and anesthetic depth. This study will use EEG SEF to monitor anesthetic depth in infants and toddlers during three stimuli (placement of oral pacifier, electrical stimulation, and laryngoscopy—a clinically required procedure), which reflect the three endpoints of clinical anesthesia depth. The median EEG SEF where 50% of patients do not respond to each of the three stimuli will be determined, along with the relationship between EEG SEF and propofol effect site concentration (Ce), as measured from plasma propofol levels at the time the EEG SEF was recorded. Results from this study should improve the safety and efficacy of propofol anesthesia in infants and toddlers by confirming EEG SEF as a biomarker of propofol anesthetic depth, thereby improving the accuracy of dosing to avoid over- and under-dosing.
Study Objective(s)	Primary In infants and toddlers receiving propofol anesthesia for a procedure determine the three EEG SEFs where 50% of patients do not respond to the three stimuli (placement of oral pacifier, electrical stimulation, and laryngoscopy—a clinically required procedure).

	Secondary In the same cohort, determine the dose-response relationship between EEG SEF and propofol Ce, as measured from plasma propofol levels.
Study Design	Sequential allocation trial using *Dixon's* up-down technique. To achieve the EEG SEF target, initial propofol dosing will be based on a published age-based dosing table used clinically at CHOP. The EEG SEF target for a specific stimulus will be determined by the previous patient's response to that same stimulus. If the previous patient responded to the stimulus, then the current patient will have a lower SEF target for the same stimulus. Conversely, if the previous patient did not respond to the stimulus, then the current patient will have a higher SEF target for the same stimulus. In the same patient, propfol dose adjustments may be needed between stimuli to achieve different SEF targets. The patient response to stimulus and EEG SEF will be recorded, along with a 1ml blood sample to determine propofol plasma level at a later date in the pharmacodynamic laboratory. The study will only be performed by the investigators listed in the study.
Patient Population key criteria for Inclusion and Exclusion:	Inclusion Criteria 1) Children 3 to 24 month old undergoing a procedure 2) Planned laryngoscopy and endotracheal intubation or LMA placement for clinical care 3) Planned propofol anesthesia for anesthesia maintenance. 4) ASA < III. 5) Muscle relaxant not indicated per planned clinical care for laryngoscopy/intubation. 6) Anticipated procedure duration approximately < 2h40min Exclusion Criteria 1) Patients undergoing an emergency procedure 2) Known severe neurological disease which might result in abnormal EEG SEF. 3) Deformities of forehead (difficult EEG sensor placement). 4) Known difficult airway. 5) Allergy to propofol.

	6) Attending anesthesiologist on record caring for patient plans to administer additional IV medication besides propofol during study phase. 7) Currently on anti-seizure medication (might alter propofol pharmacodynamics).
Number Of Patients	Up to 150 enrolled patients to produce 120 evaluable patients.
Study Duration	Each patient's participation will last from start of anesthesia to the last study stimulus.
Study Phases **Screening** **Study Laboratory**	From placement of second intravenous (IV) line or bolus of propofol (if IV is already present prior to induction) to the end of the last stimulus performed. Eligibility screening via reviewing procedure schedule and obtaining informed consent. The propofol plasma concentration will be determined in the laboratory (Institutional Core Lab at CHOP) via batch processing of collected blood samples.
Safety Evaluations	Patient safety will be monitored by adverse events (*e.g.* bronchospasm, laryngospasm) and vital signs (*e.g.* hypotension).
Statistical And Analytic Plan	The median EEG SEF (SEF_{50}) where half of the patients do not respond to a particular stimulus will be determined for each of the three stimuli. For each stimulus, each SEF crossover pair (Yes to No response or vice versa) will be treated as a SEF pair and the mean and standard deviation of all SEF pairs will determine the median SEF_{50} and standard deviation. The propofol Ce that corresponds to each SEF will be determined through the regression curve between plasma propofol concentration and SEF recorded at the time of the blood draw.
DATA AND SAFETY MONITORING PLAN	The PI will be responsible for management of data quality and ongoing assessment of safety. The PI will promptly notify the IRB of all on-site unanticipated, serious Adverse Events that are related to the research activity. Other unanticipated problems related to the research involving risk to patients or others will also be reported promptly. In addition, an independent DSMB committee: Drs. Arjunan Ganesh, Raj Subramanyam, and Tori Sutherland, who are not involved in the study will monitor and review the study progress, patient safety, and the accuracy and security of the

	emerging data. Written reports will be filed using the eIRB system in accordance with the timeline below.

1 BACKGROUND INFORMATION AND RATIONALE

1.1 Introduction

Sevoflurane inhalation and propofol intravenous are the two primary drugs to deliver general anesthesia in the pediatric population. The pharmacodynamics of sevoflurane on the brain, respiratory, and cardiovascular functions have been extensively studied and are well known.[1] The pharmacodynamics of propofol has not been well studied in the pediatric population, particularly in infants and toddlers. Consequently, propofol anesthesia in infants and toddlers is dosed without pharmacodynamic knowledge, often resulting in over- or under-dosing.[2-4] This gap in dosing knowledge is ever more relevant, with increased interest in using propofol in pediatric anesthesia due to fewer respiratory complications, less post-operative nausea vomiting and emergence delirium, compared to sevoflurane.[5-7]

Sevoflurane and propofol dosing and the resultant brain effect-site concentration (Ce) depend on the desired clinical endpoint during the conduct of anesthesia andthe procedure. The three main endpoints of surgical anesthesia are *unconsciousness*, *no response to surgical incision*, and *no response to laryngoscopy*. The dose to achieve the desired anesthetic depth for each of these three endpoints is different; the dose to unconsciousness is less than the dose for lack of response to surgical incision or laryngoscopy. Sevoflurane expired gas concentration reflects brain Ce and can be used to guide sevoflurane dosing. The sevoflurane dose and corresponding Ce to achieve unconsciousness and block response to a procedure and laryngoscopy are known in the pediatric population and differ between infants (3-12 mo), toddler (13-23 mo), and children (2-12 years).[8,9] Although the propofol dose and corresponding Ce for the same three endpoints have been established in adults and older children via laboratory measurements of propofol plasma concentration,[10,11] they remain unknown in infants and toddlers.

Propofol dosing can be guided by electroencephalography (EEG), which monitors brain electrical acitivity and reflects anesthetic depth. EEG parameters have been shown to change predictably with increasing propofol Ce in children and adults, and can be used to guide propofol dosing to the individual patient.[12,13] In adult anesthesia and in pediatric practice at CHOP, intraoperative EEG monitoring is used to guide propofol dosing and assess anesthetic depth.[14,3] Despite the advantages of using EEG to guide propofol dosing in infants and toddlers, commonly used EEG proprietary indices (*e.g.,* BIS, PSI) were developed in adults and not validated in young children. Non-proprietary EEG parameters, such as the spectral edge frequency (SEF) are valid in young children under sevoflurane, but the relationship between EEG SEF and propofol Ce have not been established in this young population. Furthermore, the relationship between EEG SEF and the three clinical endpoints (unconsciousness, lack of response to surgical incision, and lack of response to laryngoscopy) is also unknown in this age group.

The proposed study will determine the pharmacodynamics of propofol on brain, respiratory, and cardiovascular function at the three clinical endpoints in infants and toddlers receiving propofol anesthesia for a procedure. Brain, respiratory and cardiovascular function will be assessed by EEG parameters and vital signs. The propofol Ce at each of the three clinical endpoints will be assessed by measuring plasma propofol concentration, which is equal to brain concentration at steady state. Although propofol may be combined with other agents (opioids, regional anesthesia, dexmedetomidine) during anesthetic maintenance, many cases utilize propofol as the sole agent throughout the entire anesthetic (e.g., GI endoscopy, bronchoscopy, radiological procedures). The study only involves the induction period (induction to intubation), when propofol is commonly given as the sole agent, without combination of regional anesthetics, opioids, or other agents. Studies have shown that anesthetic overdose is common during the induction period (9.3%),[15] underlining the importance of determining the appropriate dosing during this period. The results of the study should improve the safety and quality of propofol anesthesia for infants and toddlers, and provide direct benefit to the study patient by detecting and preventing propofol overdose during the induction period.

1.2 Relevant Literature and Data

Propofol Anesthesia

Propofol is FDA-approved for "Induction of General Anesthesia Patients ≥ 3 years of age and Maintenance of General Anesthesia Patients ≥ 2 months of age".[16] Propofol use in the study does not involve a route of administration, dose, patient population, or other factor that significantly increases the risk (or decreases the acceptability of the risk) associated with the use.

Propofol anesthesia consist of propofol bolus and infusion, which can be combined with regional anesthetic, opioids, dexmedetomidine, and/or muscle relaxant. For this study, only propofol will be administered during the study period which lasts from placement of the second IV or first propofol dosing until the end of the last stimulus In patients with sevoflurane induction, sevoflurane will be washed out (patient expires the gas through breathing) prior to assessing EEG SEF and starting the first stimulation. Sevoflurane washout typically takes 5 minutes. After the study period (end of last stimulus), the anesthetic team on record caring for the patient can administer other medications as planned for the procedure.

EEG Monitor

The Masimo SedLine is a non-invasive 4-channel EEG monitor that is portable, easy and fast to apply to the forehead, and displays processed EEG. It is FDA-approved for use with adults and is widely used in both the adult and pediatric surgical population. We are interested in using the Spectral Edge Frequency (SEF)—a non-proprietary EEG parameter, to assess anesthetic depth. The Masimo Sedline's

usability and safety profile has been established through our studies (17-014608 and 19-016071). The safety and efficacy of EEG SEF is not being evaluated as part of this study.

EEG Parameters *vs* Anesthetic Dose

EEG waveforms can be described using amplitude (how *much* the EEG wave goes up and down) and frequency (how *fast* the wave goes up and down). Typically, with increased anesthetic dose, EEG amplitude increases and frequency decreases. Consequently, EEG spectral edge frequency (SEF), the frequency where the majority (95%) of EEG power lies, decreases reliably with increased anesthetic dose down to age 3 months.[17,18] During anesthesia and the procedure SEF is calculated and displayed on Masimo EEG monitor every second. In older children very deep propofol anesthesia (high dose), deep propofol anesthesia (moderate dose), light propofol anesthesia (low dose), and emergence (residual drug not yet cleared) correspond to SEF of <5hz, 8-11hz, 16-18hz, and >20hz, respectively.[12] In younger children (3-24mo), lighter and deeper anesthesia correspond to SEF 11.4-14.3hz and 8.1hz, respectively.[18]

EEG SEF *vs* Responses to Stimuli

During anesthesia and the procedure, anesthetic depth is assessed by the response to key stimuli. The key stimuli to assess a response in adults and children include: 1) name calling/shake-and-shout (assess consciousness); 2) electrical stimulation (a non-injurious substitute for surgical incision), and 3) laryngoscopy (assess readiness for intubation).[9,19,20] Since responses to name calling/shake-and-shout are not applicable to infants and unreliable in toddlers, the response to placement of oral pacifier is used to assess consciousness instead. Therefore, the three stimuli used in this study are: 1) placement of oral pacifier, 2) electrical stimulation, and 3) laryngoscopy—a clinically required procedure. Placement of oral pacifier involves placing a soft pacifier inside the patient's mouth. Electrical stimulation is used to assess neuromuscular function but in our study will also be used as a non-injurious substitute for surgical incision. Laryngoscopy is clinically required to place the breathing tube, regardless of the study. Since placement of the oral pacifier and electrical stimulation are stimuli that are not part of standard clinical care, only the investigators on the study will be performing the stimulations.

A positive response to each of the three stimuli is the occurrence of any of the following: swallowing, sucking, eye opening, facial movement, gross limb movement towards the source of stimulus and additionally for laryngoscopy: breath-holding, closure of the vocal cords, and coughing.[19-21] These responses typically occur at the subconscious level without causing awareness of the stimulus. If a positive response is observed, the stimulus is stopped. It is likely that a different anesthetic depth and SEF target is needed for each of the three stimuli, which can be achieved by adjusting propofol dosing. From clinical experience, in infants and toddlers, consicousness, response to surgical incision, and response to

laryngoscopy correspond to SEF of approximately >15, 10-15, < 10hz, respectively.[17] This study will determine the precise SEF for each of the 3 clinical endpoints and thereby improve propofol dosing accuracy. The standard method in anesthesia to determine dose response to clinical endpoints in patients, such as ones in our study, is the *Dixon up-down* technique.

Dixon Up-Down Technique

This technique was originally used to determine the anesthetic concentration where half of the patients did not move on surgical incision. Over the past 50 years, it has been used in anesthesia research to determine the median effective dose (ED50) for other anesthetics and stimuli, including the three indicators of anesthetic depth proposed in this study.[22-24] This sequential allocation trial method can determine pharmacodynamics using a smaller number of patients than other methods.[24]

EEG *vs* Propofol Ce

At steady state propofol plasma concentration and brain Ce are equal. This can be achieved in approximately 4-6 minutes after a dose change (2-3 *times* blood to brain half-time kinetic constant of 2 minutes) and confirmed with stable EEG SEF.[16,25] If sevoflurane was used during anesthesia induction then sevoflurane will be washed out of the patient prior to performing the first stimuli, to avoid residual sevoflurane's effects on the brain and EEG. Sevoflurane washout is accomplished by turning off sevoflurane and allowing the patient to breath out the gas, which is how sevoflurane anesthesia is discontinued in clinical care at the end of the procedure. Sevoflurane concentration measured from the expired gas of the patient is standard of care. Sevoflurane washout is complete when expired sevoflurane concentration < 0.2%. Propofol infusion and EEG monitoring will start prior to completing sevoflurane washout in order to maintain the anesthetic state while switching from sevoflurane to propofol.

Several studies used propofol plasma concentration to validate the accuracy of population pharmacokinetic models in estimating propofol Ce, including two studies in older children.[4,26-29] The first study found an inverse linear relationship between EEG BIS index (a proprietary EEG index) and propofol plasma concentration in children > 6 years old.[28] The second study found that propofol plasma concentration 3.9 mcg/ml corresponded to EEG BIS index of 50 in children 4-11 years old.[26] There are few studies looking at the association of EEG parameters and propofol plasma concentration in infants and young toddlers.

1.3 Compliance Statement

This study will be conducted in full accordance with all applicable Children's Hospital of Philadelphia Research Policies and Procedures and all applicable Federal and state laws and regulations including 45 CFR 46, 21 CFR Parts 50, 54, 56, 312, 314 and 812 and the Good

Clinical Practice: Consolidated Guideline approved by the International Conference on Harmonisation (ICH). Note: Only include the sections of Title 21 if the study is regulated by the FDA. Only include ICH compliance if the study will actually comply with these requirements. All episodes of noncompliance will be documented.

The investigators will perform the study in accordance with this protocol, obtain consent, and report unanticipated problems involving risks to patients or others in accordance with The Children's Hospital of Philadelphia IRB Policies and Procedures and all federal requirements. Collection, recording, and reporting of data will be accurate and will ensure the privacy, health, and welfare of research patients during and after the study.

2 STUDY OBJECTIVES

2.1 Primary Objective

In patients receiving propofol anesthesia fora procedure, determine the three EEG SEFs values where 50% of patients do not respond to each of the three stimuli: placement of oral pacifier, electrical stimulation, and laryngoscopy—a clinically required procedure.

2.2 Secondary Objectives

In the same cohort, determine the propofol effect site concentration (Ce), at each EEG SEF for placement of oral pacifier, electrical stimulation, and laryngoscopy, as calculated from the regression between plasma propofol levels and EEG SEF recorded at the time of the blood draw.

3 INVESTIGATIONAL PLAN

3.1 General Schema of Study Design

This is a prospective interventional study to determine EEG SEF and propofol Ce during stimuli.

3.1.1 **Screening Phase**

Potential patients will be identified from the procedure schedule using the study inclusion and exclusion criteria. Informed consent will be obtained prior to any study related procedures being performed.

3.1.2 **Study Interventional Phase**

The study interventional phase will occur during the intraoperative period from after placement of second IV line or bolus of propofol (if IV line is present prior to induction) to the last stimulus.

EEG Monitoring

EEG monitoring will start after induction and end after the last stimulus.

Stimuli

At steady-state EEG SEF, two research stimuli (placement of oral pacifier and electrical stimulation) will be performed by the study investigator(s). The third stimulus, laryngoscopy—a clinically required procedure, will be performed by the anesthesia team on record caring for the patient.

Propofol dosing

Adjustment to propofol infusion and dosing is considered standard of care and is needed to achieve different anesthetic depths and SEF targets for different stimuli. As described in the study flowchart below, if current SEF is below target SEF, then propofol dose will be decreased. Conversely, if current SEF is above target SEF, then propofol dose will be increased.

Second IV Placement

An IV separate from the propofol infusion line will be placed in order to draw blood samples to evaluate propofol plasma concentration. Often the second IV will also be clinically indicated regardless of study status. If the second IV line is unable to be placed, a butterfy needle may be used to draw a single sample.

Blood Sample Collection

Up to three 1ml blood samples, for a total of 3ml, will be collected during the study interventional phase.

3.2 Study Duration, Enrollment and Number of Sites

3.2.1 Duration of Patient Study Participation

The study duration for each patient will be the day of the procedure during the intraoperative period.

3.2.2 Total Number of Study Sites/Total Number of Patients Projected

The study will only be conducted at CHOP. It is expected that approximately 150 patients will be enrolled to produce 120 evaluable patients, 60each in the 3-12mo and 13-24mo age groups.

3.3 Study Population

3.3.1 Inclusion Criteria

1) Children 3 to 24 month old undergoing a procedure.

2) Planned laryngoscopy and endotracheal intubation or laryngeal mask airway (LMA) placement for clinical care.
3) Planned propofol anesthesia for anesthesia maintenance during study period.
4) ASA < III.
5) Muscle relaxant not indicated per planned clinical care for laryngoscopy/intubation or LMA.
6) Anticipated procedure duration approximately < 2h40min

3.3.2 Exclusion Criteria

1) Patients undergoing an emergency procedure.
2) Known severe neurological disease which might result in abnormal EEG SEF.
3) Deformities of forehead (difficult EEG sensor placement).
4) Known difficult airway.
5) Allergy to propofol.
6) Attending anesthesiologist on record caring for patient plans to administer additional IV medication besides propofol during study phase.
7) Currently on anti-seizure medication (might alter propofol pharmacodynamics).

Patients that do not meet all of the enrollment criteria may not be enrolled. Any violations of these criteria must be reported in accordance with IRB Policies and Procedures.

4 STUDY PROCEDURES

4.1 Screening Phase

Procedures below will be performed during the screening phase.

- Informed Consent/HIPAA authorization
- Medical Record Review

4.2 Study Interventional Phase

4.2.1 EEG Recording

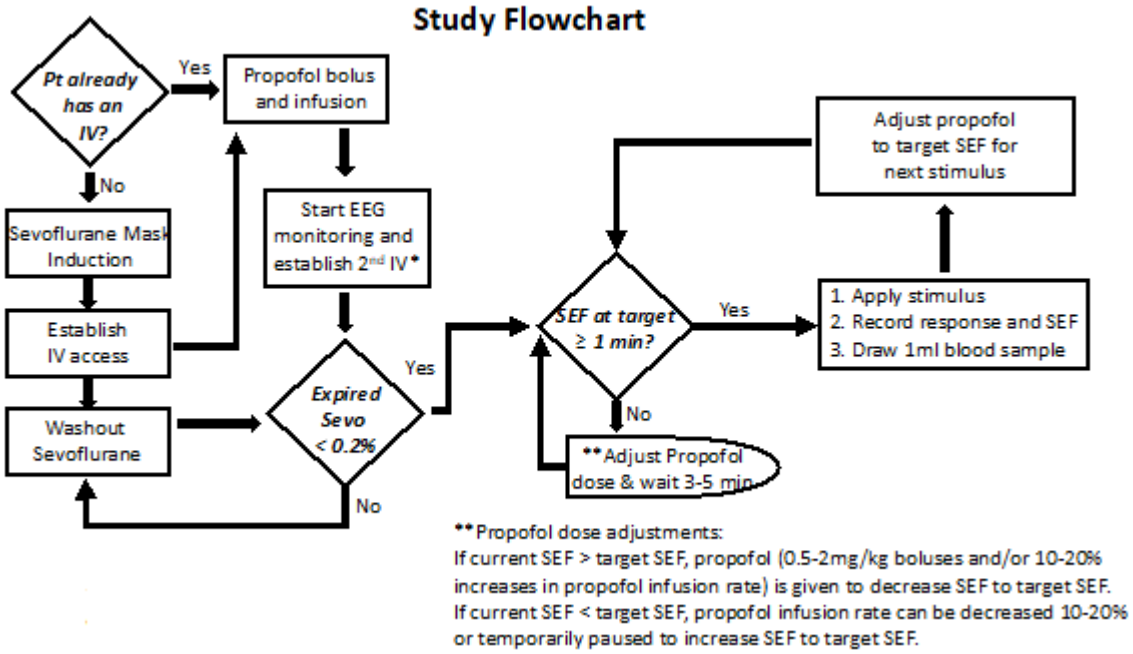
After anesthesia induction, Masimo Sedline EEG will be applied to the patient's forehead and recording will continue until after the last stimulus.

4.2.2 Stimuli

Two research stimuli: placement of oral pacifier and electrical stimulation will be performed by the study investigator. The third stimulus, laryngoscopy, will be performed by the anesthesia team on record caring for the patient. The stimulus will be performed after the patient has achieved steady state at target EEG SEF, as described in the flowchart below. The response to each of the three stimuli (Yes/No) will be recorded. Given time contraints, not all three stimuli may be performed on each patient. Each research stimulus may only be

applied once to each patient. The flowchart below shows the study procedures. Details of the stimuli are below:

1) Placement of oral pacifier involves placing a soft pacifier inside the patient's mouth.
2) Electrical stimulation is typically done to assess neuromuscular function.
3) Laryngoscopy is done prior to intubation (placement of breathing tube) and is considered a clinically-required procedure for all patients.



4.3 Patient Completion/Withdrawal

Parents or caregivers may withdraw the patient from the study at any time without prejudice to their care. They may also be discontinued from the study at the discretion of the responsible attending anesthesiologist on record and/or study investigator due to unforeseen circumstances (unable to establish IV access, lack of time, unable to achieve steady state SEF, etc…). The placement of the second IV will occur alongside routine clinical care. It will not result in additional anesthesia time as standard care is able to continue while the IV is being placed. If the study investigator becomes aware of any serious, related adverse events after the patient completes or withdraws from the study, they will be recorded in the source documents and on the CRF.

5 STUDY EVALUATIONS AND MEASUREMENTS

5.1 Screening and Monitoring Evaluations and Measurements

5.1.1 Medical Record Review

- PHI: MRN, name, element of dates including birth date and service dates
- Demographic
- Anesthetic records
- Medication list
- Surgical records
- Post operative recovery records
- Expected procedure duration and procedure type
- ASA status

5.1.2 EEG Monitoring

The study team will assess skin integrity prior to applying the EEG sensor. After anesthesia induction, the Masimo EEG sensor will be placed on the forehead and connected to the EEG monitor. EEG monitoring for the study will continue until laryngoscopy. The Masimo EEG monitor will automatically calculate and display EEG SEF values.

5.1.3 Propofol Dosing

Propofol will be administered via an infusion pump with initial dosing based on a published dosing table used in CHOP clinical practice (table 1).[12] This table serves as a starting guide to dosing, ultimately EEG SEF will be used to titrate propofol dose. Propfol dosing adjustments to achieve target SEF are described in section 5.1.8 below.

Table 1
Dosing regimens for propofol in neonates, infants, and children

Age Group	0–1 mo	1–3 mo	3–6 mo	6–12 mo	12–36 mo	3–12 y
Propofol bolus mg/kg	3.5	3	3	3	3	2.5
Propofol 0–15 min (μg/kg/min)	183	200	200	208	217	250
Propofol 16–30 min (μg/kg/min)	167	183	192	200	200	217
Propofol 31–60 min (μg/kg/min)	150	167	175	183	192	183
Propofol 61–120 min (μg/kg/min)	133	158	167	175	183	167
Propofol 121–180 min (μg/kg/min)	117	150	158	167	175	150
Propofol 181–300 min (μg/kg/min)	100	133	150	158	167	142

5.1.4 Stimuli

For this study, laryngoscopy is clinically required for anesthesia and the procedure, whereas electrical stimulation are often performed, but may not always be clincally required.

Depending on time constraints, each patient may have two to three stimuli applied in sequence (placement of oral pacifier, electrical stimulus, and laryngoscopy). Based on responses to each stimulus, the SEF target and propofol dose may be adjusted for the next

stimulus. Each stimulus may only be applied once per patient and all stimuli will be completed prior to intubation.

If sevoflurane was used during anesthesia induction, then sevoflurane will need to be washed out prior to performing the first stimuli, to avoid residual sevoflurane's effects on the brain and EEG. This can be done by turning off sevoflurane to allow the patient to expire the sevoflurae through breathing. Sevoflurane washout is complete when end tidal sevoflurane concentration < 0.2%. Propofol infusion and EEG monitoring will start prior to completing sevoflurane washout.

Placement of oral pacifier

After anesthesia induction, sevoflurane washout (if applicable), and EEG SEF at the target for placement of oral pacifier, an oral pacifier will be placed in the patient's mouth. The oral pacifier will be withdrawn if there is a positive response (defined in section 5.1.5) or when 5 seconds has elapsed.

Electrical stimulus

The study team will assess forearm skin integrity prior to applying the nerve stimulator electrodes. After sevoflurane washout (if applicable) and EEG SEF at the target for electrical stimulus, a nerve stimulator (Halyard EZstim, Halyard Health Inc) will be initiated at 50hz 30mA for up to 15 seconds.[30] The electrical stimulus will stop if there is a positive response (defined below) or when 15 seconds has elapsed.

Laryngoscopy

After sevoflurane washout (if applicable) and EEG SEF at the target for laryngoscopy, laryngoscopy will be performed by the anesthesia team on record caring for the patient. This will mark the end of the stimuli interventions. If no response to laryngoscopy is elicited, the anesthesia team on record may choose to intubate the patient or administer additional medications. If a positive response to laryngoscopy is elicited, the anesthesia team on record may choose to stop laryngoscopy and/or administer additional medications such as neuromuscular relaxant.

Responses to the stimuli will be recorded. The patient will be monitored closely by the study team for any adverse events. Study interventions will occur simultaneously with routine clinical anesthesia and surgical care; therefore, there will be no additional time under anesthesia from this study. Not every patient will have all three stimuli performed and some patients may have only one research stimuli performed. Study procedures will be stopped once all standard, clinical care preparation for surgery is completed.

The same stimulus will not be applied twice to the same patient. The patient's study involvement will conclude once laryngoscopy has been performed on the patient and blood

draw (if applicable) has been performed. The remainder of the anesthetic will be per routine care.

5.1.5 Responses to Stimuli

A positive response to the stimuli described above is occurrence of any of the following: swallowing, sucking, eye opening, facial movement, gross limb movement towards the source of stimulus, and additionally for laryngoscopy, breathing-holding, closure of vocal cords, and coughing. These responses typically occur at the subconscious level without causing awareness of the stimulus. Patient response (Yes/No) will be recorded for each stimulus applied.

5.1.6 Blood collection

Before a stimulus is applied, the SEF value and vital signs will be recorded and a 1ml blood sample will be obtained (not from the propofol infusion IV line). Up to three blood samples may be collecteds..

5.1.7 Dixon Up-Down Technique

Using *Dixon up-down* technique, the current patient's response to stimulus at a SEF target will determine the next patient's SEF target for the same stimulus, thus forming the sequential design method. Using placement of oral pacifier as an example and SEF step size of 2hz, patient #1 starts at SEF target of 18hz and has a positive response to placement of oral pacifier. Patient #2 will have a decrement of SEF target by one step size to 16hz (18 minus 2) for the same stimulus. If patient #2 also has a positive response to placement of oral pacifier, then patient #3 will have a further decrement in SEF target by one step size to 14hz (16 minus 2). If patient #3 has a negative response to placement of oral pacifier, then patient #4 will increase SEF target one step to 16hz (14 plus 2). This continues until there are consecutive crossovers (typically six up-down-up-down…), whereby the median SEF and standard deviation can be calculated for the specific stimulus.[24] Since each stimulus is of varying intensity, it's expected that each of the 3 stimuli will have its own *Dixon up-down* calculated SEF target.

5.1.8 Achieving SEF target

The EEG SEF targets will be different for each of the three stimuli (e.g., the propofol dose and anesthetic depth to block response to placement of oral pacifier is less than for electrical stimulation and laryngoscopy). Based on our clinical experience, the initial SEF targets for the first patient will be 20, 14, and 10hz for placement of oral pacifier, electrical stimulation, and laryngoscopy, respectively. For the same stimulus, the subsequent patient's target SEF will be determined by the previous patient's response to that stimulus, as

described in the section *Dixon up-down technique*. After sevoflurane washout (if applicable) and the propofol infusion dose and SEF are constant for 5 minutes, to ensure equilibration between propofol brain Ce and plasma concentration, the stimulus will be applied as described in the section *EEG vs Propofol Ce.* If current SEF is higher than target SEF, additional propofol (0.5-2mg/kg boluses and/or 10-20% increases in propfol infusion rate) can be given to decrease SEF closer to target SEF. Conversely, if current SEF is lower than target SEF, propofol infusion rate can be decreased 10-20% or temporarily paused to increase SEF to target SEF.

5.2 Laboratory test

Blood samples will be sent to Institutional Core laboratory at CHOPfor batch processing to measure plasma propofol concentration.

5.3 Safety Evaluation

Patient safety will be monitored by adverse events, vital signs, and patients' response.

5.4 Non-evaluable Subjects

Non-evaluable subjects include 1) patient received IV medication other than propofol and midazolam from the anesthesia team during study phase, 2) unable to get EEG SEF reading during study phase, or 3) unable to decrease expired sevoflurane to ≤ 0.2% during study period.

6 STATISTICAL CONSIDERATIONS

6.1 Primary Endpoint

Three EEG SEF_{50} values corresponding to where 50% of patients do not respond to 1) placement of oral pacifier EEG SEF_{50}, 2) electrical stimulation EEG SEF_{50}, and 3) laryngoscopy EEG SEF_{50}.

6.2 Secondary Endpoints

The propofol effect site concentration corresponding to each EEG SEF_{50} will be determined from the regression between plasma propofol concentration vs SEF recorded at the time of the blood draw.

6.3 Statistical Methods

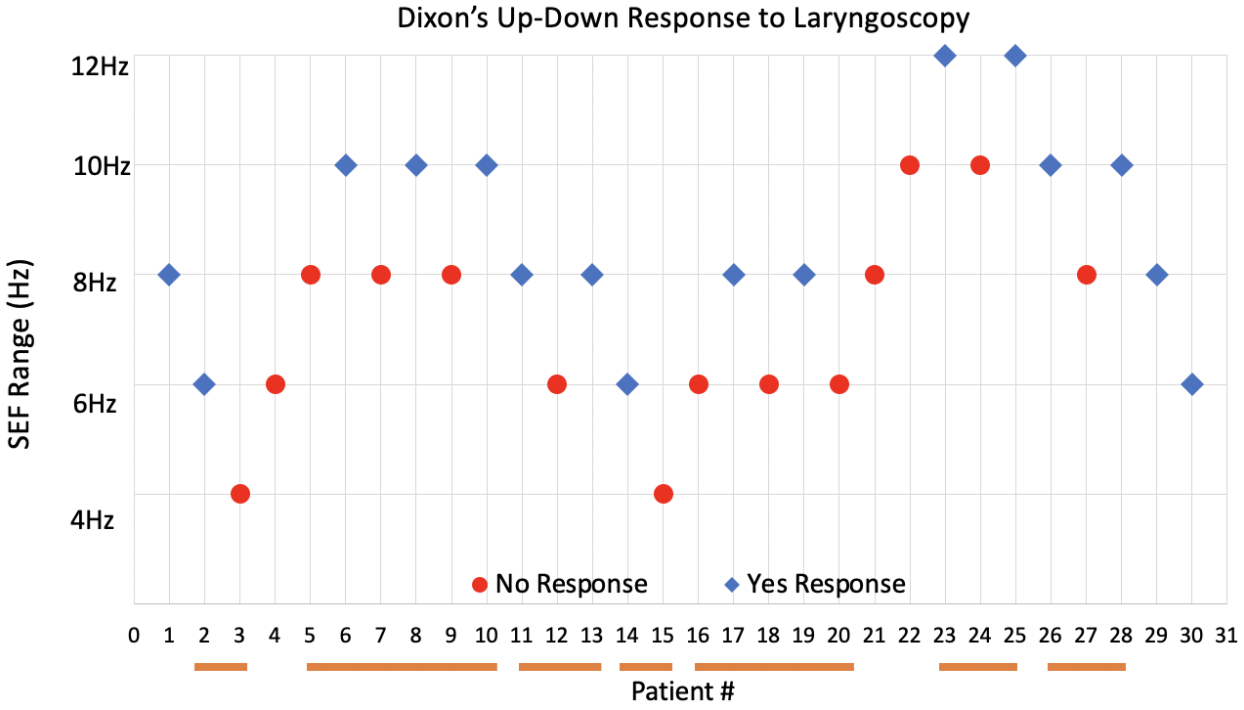
6.3.1 Baseline Data

Baseline and demographic characteristics will be summarized with standard descriptive summaries (e.g. means and standard deviations for continuous variables such as age and percentages for categorical variables such as gender).

6.3.2 **EEG SEF**

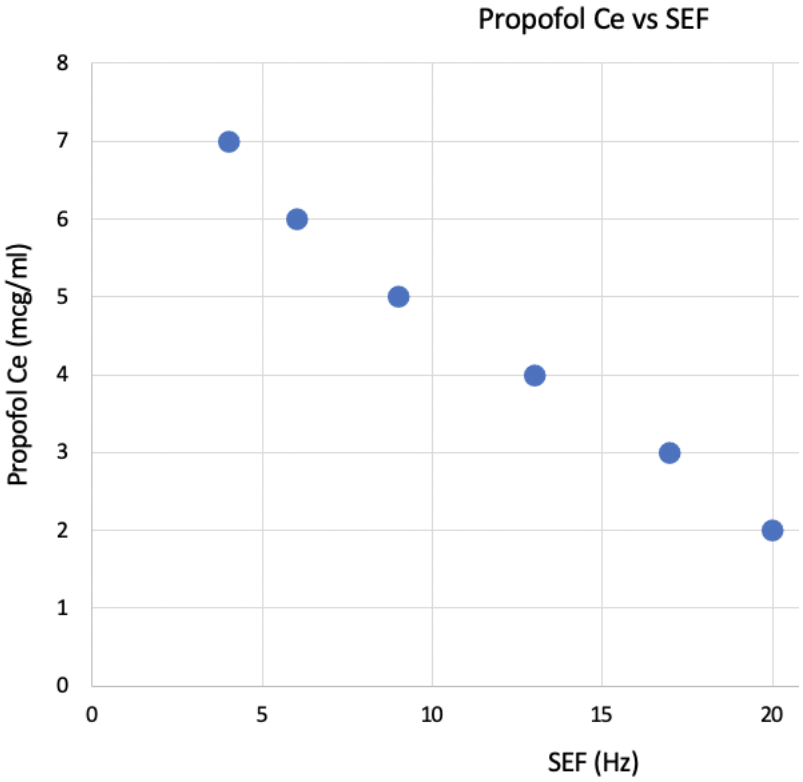
The median EEG SEF (SEF_{50}) for a stimulus is the SEF where half the patients do not respond to that particular stimulus. SEF_{50} will be determined for each of the three stimuli for the 3-12 mo and 13-24 mo age groups. For each stimuli, each SEF crossover pair (Yes to No response or vice versa) in the *Dixon's up-down* response will be treated as a SEF pair and the mean SEF_{50} and standard deviation of the SEF pairs will be determined. See figure below for example of Dixon's up-down method and determination of SEF_{50} for laryngoscopy. X axis represents sequential patients #1 to #30, Y axis represent SEF levels. Each blue (Yes response) and red (No response) dot represent a patient. The orange bars on the X axis represent crossover pairs, used to calculate SEF_{50}.

In the laryngoscopy example below, the mean SEF_{50} is 8.22hz and standard deviation is 1.83hz, derived from crossover points: 5, 9, 9, 9, 9, 9, 7, 7, 5, 7, 7, 7, 7, 11, 11, 11, 9, 9hz.



6.3.3 **Propofol Ce**

The propofol plasma concentration will be determined by laboratory measurements. A regression curve will be determined from the plot of plasma propofol concentration vs SEF at the time the blood was drawn for the propofol level. At steady state, plasma propofol and propofol Ce are equal. The propofol Ce corresponding to each SEF_{50} will be calculated from the regression. There will be two regression curves, one for the 3-12 mo age group and the other for the 13-24 mo age group. See figure below for example of Propofol Ce vs SEF curve for one age group; x-axis is SEF and y-axis is propofol Ce.



6.3.4 Sample Size and Power

Many anesthesia studies utilizing the Dixon up-down design have a sample size of 10-30 for each stimulus.[23] Hammer et al. used the *Dixon up-down* method to determine the propofol median effective dose (ED50) to prevent movement in children undergoing gastro-endoscope insertion with a sample size of 12.[31] Endoscope insertion is a procedure similar to laryngoscopy. We propose a sample size of 30 for each stimulus for each age group. Because only one or two stimuli can be performed per patient within the proposed study time (e.g., placement of oral pacifier before laryngoscopy, or nerve stimulation before laryngoscopy), the required sample size of subjectsfor each age group needs to be up to 60, for a towal of 120 evaluable patients. Two age groups are selected because of normal age-dependent EEG changes in the first few years of life that could affect the EEG SEF.

6.4 Safety Analysis

During the study period, adverse events (AE) are defined as:

1- Oxygen desaturation (< 85% for more than 30 seconds).
2- Hypotension (mean arterial pressure < 40 mmHg for 3-12 mo and < 45 mmHg for 13-24 mo across at least two measurements).
3- Bradycardia (HR < 60 bpm for more than 30 seconds) will be recorded for all patients.
4- Overhead call for emergency help.
5- Laryngospasm requiring intervention.

AE incidence will be summarized along with the corresponding SEF values during the occurrence.

7 SAFETY MANAGEMENT

7.1 Clinical Adverse Events

Clinical adverse events (AEs) will be monitored throughout the study.

7.2 Adverse Event Reporting

Unanticipated problems related to the research involving risks to patients or others that occur during the course of this study (including SAEs) will be reported to the IRB in accordance with CHOP IRB SOP 408: Unanticipated Problems Involving Risks to Patients. AEs that do not meet prompt reporting requirements will be summarized in narrative or other format and submitted to the IRB at the time of continuing review (if continuing reviews are required), or will be tracked and documented internally by the study team but not submitted to the IRB (if continuing reviews are not required).

7.3 Definition of an Adverse Event

An adverse event is any untoward medical occurrence in a patient who has received the study intervention procedures: placement of oral pacifier and electrical stimulation. The occurrence does not necessarily have to have a causal relationship with the treatment. An AE can therefore be any unfavorable or unintended sign, symptom, or disease temporally associated with the use of study required procedure, whether or not considered related to the study procedure.

All AEs (including serious AEs) will be noted in the study records and on the case report form with a full description including the nature, date and time of onset, determination of non-serious versus serious, intensity (mild, moderate, severe), duration, causality, and outcome of the event.

7.4 Definition of a Serious Adverse Event (SAE)

An SAE is any adverse drug experience occurring at any dose that results in any of the following outcomes:

- death,
- coma,
- heart, lung, or brain demage,
- requires unanticipated inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant disability/incapacity, or an important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a

serious adverse event when, based upon appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

7.4.1 Relationship of SAE to study intervention

The relationship of each SAE to the study intervention should be characterized using one of the following terms in accordance with CHOP IRB Guidelines: definitely, probably, possibly, unlikely or unrelated.

7.5 IRB/IEC Notification of SAEs and Other Unanticipated Problems

The Investigator will promptly notify the IRB of all on-site unanticipated, serious Adverse Events that are related to the research activity. Other unanticipated problems related to the research involving risk to patients or others will also be reported promptly. Written reports will be filed using the eIRB system and in accordance with the timeline below. External SAEs that are both unexpected and related to the study intervention will be reported promptly after the investigator receives the report.

Type of Unanticipated Problem	Initial Notification (Phone, Email, Fax)	Written Report
Internal (on-site) SAEs Death or Life Threatening	24 hours	Within 2 calendar days
Internal (on-site) SAEs All other SAEs	7 days	Within 7 business days
Unanticipated Problems Related to Research	7 days	Within 7 business days
All other AEs	N/A	Brief Summary of important AEs may be reported at time of continuing review

7.5.1 Follow-up report

If an SAE has not resolved at the time of the initial report and new information arises that changes the investigator's assessment of the event, a follow-up report including all relevant new or reassessed information (e.g., concomitant medication, medical history) should be submitted to the IRB. The investigator is responsible for ensuring that all SAE are followed until either resolved or stable.

8 STUDY ADMINISTRATION

8.1 Data Collection and Management

Minimal identifiable information might be printed and recorded on paper for study eligibility clarification, study data collection, data validation, and consenting document. Any paper documents will only be accessible to approved CHOP staff and will be kept in the locked

research office when not in use. Any un-needed paper data that has identifiable information will be shredded as soon as possible.

- Confidentiality: A master list containing PHI and patient ID number will be kept in REDCap. PHI will be labled as "Identifier" in REDCap, so data could be exported as coded dataset for analysis.
- Security: Data will be kept in the REDCap database and CHOP secure servers.

8.2 Confidentiality

No identifiable data will be used for future study without first obtaining IRB approval or determination of exemption. All data and records generated during this study will be kept confidential in accordance with Institutional policies and HIPAA on subject privacy and that the Investigator and other site personnel will not use such data and records for any purpose other than conducting the study. The investigator will obtain a data use agreement between the provider (the PI) of the data and any recipient researchers before sharing a limited dataset (PHI limited to dates and zip codes).

8.3 Regulatory and Ethical Considerations

8.3.1 Data and Safety Monitoring Plan

The study will have an independent DSMB committee: Drs. Arjunan Ganesh, Raj Subramanyam, and Tori Sutherland, who are not involved in this study. The DSMB committee will meet before every continuing review or as needed to monitor and review the study progress, patient safety, and the accuracy and security of the emerging data. During the study period, each patient will be monitored for any adverse events or safety concerns by investigators. Additionally, the PI will monitor and review the study progress, subject safety, and the accuracy and security of the emerging data.

8.3.2 Risk Assessment

The assessment to support the study involving greater than minimal risk but presenting the propect of direct benefit to the individual subjects to qualify for approval under 45 CFR §46.405. The study protocol required procedures are placement of oral pacifier, electrical stimulus, laryngoscopy, and blood draws from an IV.

- Risks of placement of oral pacifier: Minimal.
- Risks of electrical stimulation: Minimal (redness and irritation to the skin from the adhesive on the electrodes, which should self-resolve within 24 hours).
- Risks of laryngoscopy: Coughing, breath-holding, vocal cord closure, and arterial desaturation. Laryngoscopy is a clinically necessary procedure that will be performed regardless of study. Patients may have increased risk of vocal cord closure because they will not receive a muscle relaxant for intubation, although laryngoscopy is often done without muscle relaxant in standard clinical care (55% of the time). Furthermore, a muscle relaxant for intubation is contraindicated for many patients in our study due to

the type of procedure. Patients undergoing a procedure who are not expected to require muscle relaxant will be enrolled. Patients may experience temporary arterial desaturation during anesthesia care related to coughing and breathholding during induction, emergence, and recovery. Laryngoscopy is not a study-related risk and will occur regardless of study participation.

- Risks of blood draws: Minimal. Blood will be collected while the patient is anesthetized. The investigator will adhere to the NIH "Guidelines for Limits of Blood Drawn for Research Purposes" and no more than 5 mL/kg will be drawn for research purposes in a single day.

The study period occurs before the procedure and any surgical stimulation, therefore there is no risk associated with withholding regional anesthetic, opioids, or dexmedetomidine. Propofol is often given as the sole agent during the induction period. There may be a small risk associated with withholding muscle relaxant prior to laryngoscopy, The potential risks include coughing, movement, and laryngospasm, although these risks are far less with propofol.

Study interventions will happen concurrenly with routine clincal care; there will be no additional anesthesia time.

The risks associated with the use of EEG are no more than minimal. It is possible that the adhesive sensor may irritate the skin. Sensor sites will be checked for possible irritation.

8.3.3 **Potential Benefits of Trial Participation**

There may be direct benefits to the study participants, since they will have EEG monitoring during the study period making them less likely to experience isoelectric EEG and associated hypotension. Isoelectric EEG is common in young children under anesthesia, 63% at CHOP[32] and 32% worldwide[15]. Isoelectric EEG is associated with intraoperative moderate and severe hypotension, preventable critical events that increase morbidity.[33] Indirect benefits to future patients include: 1) improved accuracy of propofol dosing in infants and toddlers; 2) safer propofol anesthesia care- lower incidence of hypotension from over-dosing, and lower incidence of insufficient anesthesia from propofol under-dosing; and 3) better efficacy- faster emergence and recovery from less propofol over-dosing.

8.3.4 **Risk-Benefit Assessment**

This study involves no more than a minor increase above minimal risk. There are direct benefits to study participants to have EEG monitoring to prevent isoelectric EEG and associated hypotension, and indirect benefits to future patients for preventing propofol under- and over-dosing.

8.4 Recruitment Strategy

Potential patients will be identified from the scheduled procedure list. Investigator will only approach permissible patients.

8.5 Informed Consent and HIPAA Authorization

Patients's parent(s)/legal guardian(s) will be approached by investigator prior to the procedure in a private setting, such as a private exam room. The investigation team will make a good faith effort to obtain consent the day prior to the procedure. The primary aim, risks, benefits and study procedures for the study will be explained and the parent(s)/legal guardian(s) will have ample time and opportunity to ask questions. It will be reiterated that the decision to participate or decline to participate in the study will have no bearing on their medical care.

Per institutional policy, the investigators intend to obtain electronic "written" consent via REDCap, the following criteria apply

- A valid electronic signature must be obtained.
- The patient will receive an electronic copy of completed consent and HIPAA authorization form by emailing from REDCap.

If unable to obtain consent electronically, a paper copy of the consent form will be signed and uploaded to the study's REDCap.

Individuals with Limited English Proficiency

The short form consent process will be utilized for enrollement of limited English proficiency patients. The investigator is familiar with the IRB's guidance on the short form consent process, i.e. the use of interpreters, the requirement of a witness, and the required signing of a study summary document and a short form consent form by the respective parties. If REDCap electronic consent is used for LEP patients and an in-person interpreter is not available, phone interpretation through Language Services Associates will be utilized as outlined in the Office of Research Compliance guidance and no study procedures will take place until all electronic signatures have been obtained.

8.6 Payment to Patients/Families

There will be no payments to families.

9 PUBLICATION

Publication in a peer-reviewed journal will be pursued after completion of the study. Only aggregate data without individual identifiable information will be published.

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