Cryoanalgesia vs. Epidural in the Nuss Procedure
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LIST OF ABBREVIATIONS

AE	adverse event
CFR	Code of Federal Regulations
CRF	case report form
DMC	Data Monitoring Committee
DSMB	Data Safety Monitoring Board
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act of 1996
ICF	informed consent form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IV	intravenous
mEq	milliequivalent
PI	Principal Investigator
SAE	serious adverse experience

PROTOCOL SYNOPSIS

TITLE	Cryoanalgesia vs. Thoracic Epidural for Post-operative Pain Control following the Nuss Procedure
SPONSOR	UCSF Department of Surgery, Division of Pediatric Surgery
NUMBER OF SITES	1: UCSF Benioff Children's Hospital
RATIONALE	Pectus excavatum is the most common congenital chest wall deformity, affecting approximately 1 out of every 1000 live births. It presents as a depression of the anterior chest wall, which can compress the heart and lungs, causing symptoms such as shortness of breath, exercise intolerance, and chest pain. The minimally invasive pectus excavatum repair, known as the Nuss procedure, is a common corrective procedure. It uses an implanted, thoracoscopically-guided convex metal bar to immediately reshape the chest wall. The benefits of this method compared to open repair include smaller incisions, no cartilage resection or osteotomy, and reduced operative times. However, the immediate reshaping of the chest wall results in significant post-operative pain, which can be challenging for patients, families, and clinicians. The ability to adequately control this post-operative pain is the primary determining factor for length of hospitalization following the procedure. There is no standardized protocol for the management of pain following the Nuss procedure. Epidural analgesia and patient controlled analgesia (PCA) are the two most commonly used techniques, employed either independently or together. While epidural analgesia has been shown to be more effective than PCA alone for the first few postoperative days, it carries significant risks and is limited to the first two to three post-operative days, at which time patients must be transitioned to alternate methods. Cryoanalgesia is a means of long-term local nerve blockade, which has previously been used in adults for acute pain control following thoracotomy, as well as for treatment of chronic thoracic pain due to surgery or post-herpetic neuralgia. Anecdotally, cryoanalgesia has been effective at controlling acute post-operative pain following the Nuss procedure. The procedure is particularly well-suited to the application of cryoanalgesia, as the operative exposure directly visualizes the intercostal nerves. However, no randomized trials have been performed to more rigorously evaluate this treatment. We therefore propose a randomized, prospective trial to evaluate cryoanalgesia compared to epidural analgesia for the Nuss procedure.
STUDY DESIGN	This study is a prospective, randomized trial to compare cryoanalgesia to standard epidural analgesia for post-operative pain management in patients undergoing minimally invasive pectus excavatum repair (the Nuss procedure). 20 subjects will be randomized in 1:1 ratio to receive cryoanalgesia or epidural analgesia prior to their procedure.
PRIMARY OBJECTIVE	To compare post-operative length of stay in patients receiving cryoanalgesia vs. thoracic epidural analgesia for the Nuss procedure

SECONDARY OBJECTIVES	To compare post-operative pain scores, pain medication usage, side effects, and total cost of hospital stay in patients receiving cryoanalgesia vs. thoracic epidural analgesia for the Nuss procedure
NUMBER OF SUBJECTS	20
SUBJECT SELECTION CRITERIA	Inclusion Criteria: Otherwise healthy male or female patients with pectus excavatum deformity, scheduled for Nuss procedure for chest wall correction, at least 13 years of age at time of Nuss procedure. Exclusion Criteria: • Age less than 13 years at time of surgery • Pre-op pain medication usage • Pectus carinatum • Poland's syndrome or other complex chest wall anomaly • Previous repair of Pectus Excavatum by any technique • Previous thoracic surgery • Congenital heart disease • Bleeding dyscrasia • History of previous problems with anesthesia or major anesthestic risk factors • Pregnancy • Non-English speaker
TEST PRODUCT, DOSE, AND ROUTE OF ADMINISTRATION	AtriCure cryosurgical system (AtriCure, Inc., West Chester, Ohio) The cryoprobe will be applied to the intercostal nerve under thoracoscopic visualization at the level of the incision and 2 interspaces above and below, bilaterally. The temperature and duration of cryoablation therapy is pre-set by the probe and cryosurgical system at 2 minutes to -60°C. Each nerve will receive one cycle of cryoablation.
CONTROL PRODUCT, DOSE AND ROUTE OF ADMINISTRATION	Thoracic epidural catheter will be placed by the pediatric anesthesia team prior to Nuss procedure. Catheters will be placed using sterile technique at approximately T5-6 or T6-7 interspaces; procedure specifics are dependent on the attending anesthesiologist's patient-dependent preference. Whether the patient will be awake or asleep for epidural placement will be at the discretion of the attending anesthesiologist. After placement, epidural infusion will begin with 0.1% ropivicaine and 2mcg/cc fentanyl. No bolus doses will be used.

DURATION OF SUBJECT PARTICIPATION AND DURATION OF STUDY	Subjects will be on study for 1 year **Screening:** 1 week (prior to initial consultation) **Treatment:** 3-7 days (subjects to be admitted to the hospital) **Follow-up:** 1 year The total duration of the study is expected to be 1.5 years. 6 months for subject recruitment and 1 year following final recruitment for subject follow-up.
CONCOMMITANT MEDICATIONS	Allowed: all deemed necessary by primary team or pediatric pain service Prohibited: none
EFFICACY EVALUATIONS	
PRIMARY ENDPOINT	• Post-operative hospital length of stay
SECONDARY ENDPOINTS	• Post-operative pain scores • Pain medication usage • Side effects • Total hospitalization cost
SAFETY EVALUATIONS	Incidence of adverse events
PLANNED INTERIM ANALYSES	Serious adverse events will be monitored by the committee on an ongoing basis throughout the study.
STATISTICS **Primary Analysis Plan**	Patient baseline characteristics will be analyzed with descriptive statistics (ie. mean age, % male, mean pectus severity index, etc.). Our primary outcome will be post-operative length of stay, which will be analyzed using a two-tailed unpaired t test, with results considered statistically significant for $p< 0.05$. Secondary outcomes include post-operative pain scores, pain medication usage, and side effects. Pain scores will be analyzed with Mann-Whitney rank sum tests (to compare between groups at each time point) and Wilcoxson signed-rank test (to compare scores within groups over time), with results considered statistically significant for $p< 0.05$.. Pain medication usage between the two groups will be analyzed with two-tailed unpaired t test, again with results considered statistically significant for $p< 0.05$. Given our small sample size and the low rate of complications with both analgesic methods, we expect very limited side effect data. A descriptive overall side effect rate will be calculated for each arm of the study, and the individual side effects will be listed in a table. A cost analysis will be performed, comparing the total cost of hospitalization between the cryoanalgesia and epidural groups. Difference in total cost will be assessed with two-tailed unpaired t test.

Rationale for Number of Subjects	We used the UCSF Clinical & Translational Science Institute web-based sample size calculator (http://www.sample-size.net/sample-size-means/) to determine study size. Based on previous Nuss procedures over the past 2 years at our institution, we determined the mean length of hospitalization was 7 days, with a standard deviation of 1.3 days. From anecdotal data from our prior experience, as well as from speaking with other surgeons utilizing cryoablation, we expect patients in the cryoanalgesia group to have approximately 2 days shorter hospital stay. With type 1 error rate of 0.05 and type II error rate of 0.1 (yielding 90% power), our study would need 18 patients total. Allowing for some dropout, we will plan to enroll 20 patients to the study.

1 BACKGROUND

Pectus excavatum is the most common congenital chest wall deformity, affecting approximately 1 out of every 1000 live births. It presents as a depression of the anterior chest wall, which can compress the heart and lungs, causing symptoms such as shortness of breath, exercise intolerance, and chest pain.[1,2] The minimally invasive pectus excavatum repair, known as the Nuss procedure, is a common corrective procedure. It uses an implanted, thoracoscopically-guided convex metal bar to immediately reshape the chest wall.[1,3,4] The benefits of this method compared to open repair include smaller incisions, no cartilage resection or osteotomy, and reduced operative times. [3] However, the immediate reshaping of the chest wall results in significant post-operative pain, which can be challenging for patients, families, and clinicians.[5,6] The ability to adequately control this post-operative pain is the primary determining factor for length of hospitalization following the procedure.[7]

There is no standardized protocol for the management of pain following the Nuss procedure. Epidural analgesia and patient controlled analgesia (PCA) are the two most commonly used techniques, employed either independently or together. [5-8] Thoracic epidural analgesia has been shown, with limited data, to be more effective than PCA alone for the first few postoperative days, but carries significant risks, including nerve damage, infection, and respiratory depression. [6,9] Epidural analgesia also reduces post-operative mobility, and often necessitates the use of urinary catheters, which can increase risk of urinary tract infections.[10] The use of epidural analgesia is limited to the first two to three post-operative days, at which time patients must be transitioned to alternate methods.[11] This may increase length of stay, as it introduces an intermediate step prior to home oral regimen.

Thus, there is significant room for improvement in post-operative pain management in the Nuss procedure. One possible solution is the addition of a local nerve block with cryoanalgesia. The use of cold to numb pain is one of the oldest forms of analgesia, dating back at least to Hippocrates (460-377 BC), who described the use of ice and snow packs for relieving surgical pain.[12] Today, the term "cryoanalgesia" refers to the localized freezing of peripheral nerves. Freezing occurs through application of a "cryoprobe," which releases high-pressure carbon dioxide or nitrous oxide that rapidly expands, causing cooling via the Joule-Thomson effect to temperatures of -50 to -70 °C. This sudden freezing causes the formation of intra-and extracellular ice crystals, which disrupt the cell wall and alter cellular osmolality. When nerve axons are frozen, Wallerian degeneration occurs, preventing the transmission of pain signals and providing analgesia. However, the fibrous neural structures, including the perineurium and epineurium, are able to resist damage and remain intact, which facilitates axonal regeneration. [10,12-14] The rate of this axonal regeneration is approximately 1-3 mm/day. Animal studies of cryoanalgesia have demonstrated that repair and regeneration of the axon and myelin sheath following cryoanalgesia is complete by approximately 4-6 weeks.[10,15]

Cryoanalgesia has previously been used in adults for acute pain control following thoracotomy, as well as for treatment of chronic thoracic pain due to surgery or post-herpetic neuralgia.[10,11,16-25] Anecdotally, cryoanalgesia has been effective at controlling acute post-operative pain in young adult and adult patients following the Nuss procedure. The procedure is particularly well-suited to the application of cryoanalgesia, as the operative exposure directly visualizes the intercostal nerves. However, no randomized trials have been performed to more rigorously evaluate this treatment. We therefore propose a randomized, prospective trial to evaluate cryoanalgesia compared to epidural analgesia for the Nuss procedure.

1.1 Overview of Non-Clinical Studies

Scientists have been investigating the effect of cold on the nervous system since approximately 400 BC. Since the 1970s, "cryoanalgesia" systems have been in use, which deliver

high-pressure carbon dioxide or nitrous oxide that rapidly expands, causing cooling via the Joule-Thomson effect to temperatures of -50 to -70 °C. This sudden freezing causes the formation of intra-and extracellular ice crystals, which disrupt the cell wall and alter cellular osmolality. When nerve axons are frozen, Wallerian degeneration occurs, preventing the transmission of pain signals and providing analgesia. However, the fibrous neural structures, including the perineurium and epineurium, are able to resist damage and remain intact, which facilitates axonal regeneration. [10,12-14] The rate of this axonal regeneration is approximately 1-3 mm/day. Animal studies of cryoanalgesia have demonstrated that repair and regeneration of the axon and myelin sheath following cryoanalgesia is complete by approximately 4-6 weeks.[10,15]

1.2 Overview of Clinical Studies

There is no published literature on cryoanalgesia in pectus excavatum repair. Studies investigating pain control following the Nuss procedure shown, with limited data, thoracic epidural analgesia to be more effective than PCA alone for the first few postoperative days. However, this method carries significant risks, including nerve damage, infection, and respiratory depression.[6,9] Epidural analgesia also reduces post-operative mobility, and often necessitates the use of urinary catheters, which can increase risk of urinary tract infections.[10] The use of epidural analgesia is limited to the first two to three post-operative days, at which time patients must be transitioned to alternate methods.[11] This may increase length of stay, as it introduces an intermediate step prior to home oral regimen.

Cryoanalgesia has previously been used in adults for acute pain control following thoracotomy, as well as for treatment of chronic thoracic pain due to surgery or post-herpetic neuralgia.[10,11,16-25] Anecdotally, cryoanalgesia has been effective at controlling acute post-operative pain in young adult and adult patients following the Nuss procedure and has led to shorter post-operative length of stay.

2 STUDY RATIONALE

As above, there is significant room for improvement in post-operative pain management in the Nuss procedure. The procedure is particularly well-suited to the application of cryoanalgesia, as the operative exposure directly visualizes the intercostal nerves. The majority of patients who undergo the Nuss procedure are teens and young adults. This is because patients often become symptomatic from pectus excavatum at this age. Moreover, the younger the patient, the more compliant the chest wall, allowing for effective remodeling. Because this procedure is most commonly performed in this age-group, it is important for this study to include these patients to ensure the data is applicable to the pertinent patient population.

2.1 Risk / Benefit Assessment

We believe that cryoanalgesia may provide better post-operative pain control following the Nuss procedure than the current standard therapy, thus allowing patients to return home sooner after surgery. This randomized study will demonstrate whether this hypothesis is valid. If so, it will provide convincing data to encourage other surgeons to adopt this new method, benefitting future patients who undergo the Nuss procedure by decreasing the significant pain associated with the procedure. Furthermore, the post-operative pain and long hospital stay are often frightening for patients considering the procedure, and knowing that the post-operative course is less arduous may make patients more comfortable prior to surgery. Shortening the post-operative hospital stay following Nuss procedure may benefit society by reducing the resources allocated to this procedure; the cost analysis included in the study will further define the possible cost savings.

Even if this study shows there is no benefit with cryoanalgesia, and, in fact, the current standard thoracic epidural analgesia is preferred, this is also important information for physicians

performing the procedure or caring for these patients post-operatively, as well as for patients themselves.

There are few additional risks to patients specifically from taking part in the study. The risks above, with the exception of the risk of randomization, are all associated either with the procedure itself, or one of the methods of pain control, both of which are being offered outside of this study. By systematically studying these two methods, we hope to provide definitive data on the preferred post-operative pain control that can inform future patient and physician decisions.

3 STUDY OBJECTIVES

3.1 Primary Objective

In performing this study, we hope to determine whether cryoanalgesia can help patients recover more quickly from the Nuss procedure than standard epidural analgesia.

3.2 Secondary Objectives

We also hope to see if there is a difference in post-operative pain, medication usage, side effects, and total hospitalization cost between the two anesthetic methods.

4 STUDY DESIGN

4.1 Study Overview

This study is a prospective, randomized trial to compare cryoanalgesia to standard epidural analgesia for post-operative pain management in patients undergoing minimally invasive pectus excavatum repair (the Nuss procedure). Approximately 20 subjects will be randomized in 1:1 ratio to receive cryoanalgesia or epidural analgesia at the time of their procedure. Post-operative care and analgesic protocol will be identical for all patients with the exception of continuous epidural infusion (no bolus or PCEA). Upon discharge, patients will return to clinic for 4 total follow-up appointments: 2 weeks, 1 month, 3 month, and 1 year from the date of their surgery.

Screening data will be reviewed to determine subject eligibility. Subjects who meet all inclusion criteria and none of the exclusion criteria will be entered into the study.

Total duration of subject participation will be 1 year. Total duration of the study is expected to be 1.5 years.

5 CRITERIA FOR EVALUATION

5.1 Primary Efficacy Endpoint

Hospital length of stay, measured from time of surgery to time of discharge

5.2 Secondary Efficacy Endpoints

- Post-operative pain scores
- Post-operative pain medication usage
- Side effects
- Total cost of hospitalization

5.3 Safety Evaluations

- Incidence of adverse events, including intractable pain, analgesic failure, infection, respiratory depression, need for further intervention, or anything else deemed by the study team to fit the description of an adverse event. Adverse events will be assessed in terms of relationship to anesthetic method, relationship to procedure, severity, subsequent treatment/intervention required, and resolution status.

6 SUBJECT SELECTION

6.1 Study Population

Subjects with a diagnosis of pectus excavatum, undergoing Nuss procedure who meet the inclusion and exclusion criteria will be eligible for participation in this study.

6.2 Inclusion Criteria

1. Male or female ≥ 13 years of age at time of surgery.
2. Documentation of a pectus excavatum as evidenced by Haller index > 3.25.
3. Written informed consent (and assent when applicable) obtained from subject or subject's legal representative and ability for subject to comply with the requirements of the study.

6.3 Exclusion Criteria

- Pre-op pain medication usage
- Pectus carinatum
- Poland's syndrome or other complex chest wall anomaly
- Previous repair of Pectus Excavatum by any technique
- Previous thoracic surgery
- Congenital heart disease
- Bleeding dyscrasia
- History of previous problems with anesthesia or major anesthestic risk factors
- Pregnancy or active breastfeeding
- Non-English speaker

7 CONCURRENT MEDICATIONS

All subjects should be maintained on the same medications throughout the entire study period, as medically feasible, with no introduction of new chronic therapies.

7.1 Allowed Medications and Treatments

Standard therapy is allowed. Patients on pain medication prior to procedure will be excluded, as stated above.

Prohibited Medications and Treatments: none

8 STUDY TREATMENTS

8.1 Method of Assigning Subjects to Treatment Groups

Prior to enrollment, the patients will be randomly assigned to cryoanalgesia or thoracic epidural groups in a 1:1 ratio using a computerized random sequence generator (http://www.random.org/). The values 1-20 are entered in the system, which creates a random sequence of the integers, formatted in 2 columns of 10 integers each. Column 1 is assigned to cryoanalgesia, and column two is assigned to thoracic epidural. The assignments are placed in closed envelopes with the integers on the outside of the envelopes. The study surgeons are blinded to the randomization. As patients are enrolled in the study, they are assigned an integer in order of enrollment (the first patient enrolled is patient 1, the next patient 2, etc.). The envelope associated with each patient number is opened on the morning of surgery, revealing their group assignment.

8.2 Blinding

Due to the nature of the study, it is not possible to blind patients or clinicians to the method of analgesia once the patient's group is revealed. However, to prevent selection bias, the study clinicians and patients will be blinded to the randomization process and selection until the time of surgery.

8.3 Test and Control Products

8.3.1 Test Product

AtriCure cryosurgical system (AtriCure, Inc., West Chester, Ohio)

The cryoprobe will be applied to the intercostal nerve under thoracoscopic visualization at the level of the incision and 2 interspaces above and below, bilaterally. The temperature and duration of cryoablation therapy is pre-set by the probe and cryosurgical system at 2 minutes to -60°C. Each nerve will receive one cycle of cryoablation.

8.3.2 Control Product

Thoracic epidural catheter will be placed by the pediatric anesthesia team prior to Nuss procedure. Catheters will be placed using sterile technique at approximately T5-6 or T6-7 interspaces; procedure specifics are dependent on the attending anesthesiologist's patient-dependent preference. Whether the patient will be awake or asleep for epidural placement will be at the discretion of the attending anesthesiologist. After placement, epidural infusion will begin with 0.1% ropivicaine and 2mcg/cc fentanyl. No bolus doses will be used.

8.4 Supply of Study Drugs and Equipment at the Site

All study drugs and equipment are regularly stocked in the hospital. Prior to each Nuss procedure, both the AtriCure Cryosurgical system and supplies for thoracic epidural will be made available in the operating room.

8.4.1 Post-operative Pain Regimen

Please see separate post-operative pain regimen document

8.4.2 Storage

Medication for epidural administration and post-operative pain regimen should be stored by the study site at controlled room temperature, 15 to 30ºC (59 to 86ºF). If the temperature of study drug

storage in the clinic/pharmacy exceeds or falls below this range, this should be reported to the Sponsor or study coordinator and captured as a deviation. All medications will be stored in the hospital pharmacy and dispensed by the patient's nurse. On discharge, subjects will be instructed to store the medication in original packaging at room temperature according to the instructions outlined on the Drug Administration Instructions.

8.5 Study Drug Accountability

An accurate and current accounting of the dispensing and return of study drug for each subject will be maintained on an ongoing basis by a member of the study site staff. The number of study drug dispensed and returned by the subject will be recorded on the Investigational Drug Accountability Record. The study monitor will verify these documents throughout the course of the study.

8.6 Measures of Treatment Compliance

Subjects will be asked to keep a patient diary noting the day and date they take their pain medications and any adverse events. They will be asked to bring their patient diary to each study visit along with all used and unused study drug containers.

9 STUDY PROCEDURES AND GUIDELINES

A Schedule of Events representing the required testing procedures to be performed for the duration of the study is diagrammed in Appendix 1.

Prior to conducting any study-related activities, written informed consent and the Health Insurance Portability and Accountability Act (HIPAA) authorization must be signed and dated by the subject (if legally able to consent) or subject's parents or guardian(s). If appropriate, assent must also be obtained prior to conducting any study-related activities.

9.1 Clinical Assessments

9.1.1 Concomitant Medications

All concomitant medication and concurrent therapies will be documented at initial clinic visit/screening, during hospitalization, and at follow-up visits. Dose, route, unit frequency of administration, and indication for administration and dates of medication will be captured.

9.1.2 Demographics

Demographic information (date of birth, gender, race) will be recorded at screening.

9.1.3 Medical History

Past medical history and information regarding underlying diseases will be recorded at screening.

9.1.4 Physical Examination

A complete physical examination will be performed by either the investigator or a subinvestigator who is a physician at the initial clinic visit. Qualified staff (MD, NP, RN, and PA) may complete the abbreviated physical exam at all other visits. New abnormal physical exam findings must be documented and will be followed by a physician or other qualified staff at the next scheduled visit.

9.1.5 Vital Signs

Body temperature, blood pressure, pulse and respirations will be performed at all clinic visits and according to nursing unit protocol while patients are hospitalized.

9.1.6 Adverse Events

Information regarding occurrence of adverse events will be captured throughout the study. Duration (start and stop dates), severity, outcome, treatment and relation to study drug will be recorded on the case report form (CRF).

10 EVALUATIONS BY VISIT

10.1 Visit 1 – Pre-operative evaluation

1. Review the study with the subject (subject's legal representative) and obtain written informed consent and HIPAA authorization and assent, if appropriate.
2. Record demographics data.
3. Record medical history, including a history of pectus excavatum and any prior treatments or procedures.
4. Record concomitant medications.
5. Perform a complete physical examination.
6. Perform and record vital signs.
7. Perform and record oximetry.
8. Perform and record results of blood pressure testing.
9. Assign unique study number for randomization

10.2 Visit 2- Surgery and Post-operative hospitalization

Time of Surgery

1. Urine pregnancy test (females)
2. Open randomization allocation envelope
3. Initial analgesic application (cryoanalgesia or epidural) and Nuss procedure

According to inpatient unit protocol

1. Perform and record vital signs.
2. Perform and record oximetry.

Once per day while inpatient

1. Concomitant medications review.
2. Perform abbreviated physical examination.

Twice-per-day study visits while inpatient

1. Record any Adverse Experiences
2. Administer inpatient survey (see separate document)

10.3 Visit 3- 2 weeks post-procedure

1. Record any Adverse Experiences and Review subject diary for adverse experiences and dosing compliance.
2. Record changes to concomitant medications.
3. Perform abbreviated physical examination.
4. Perform and record vital signs.
5. Perform and record oximetry.
6. Perform post-op survey (see separate document for study tool).
7. Review pill bottles for medication usage.

10.4 Visit 4 – 1 month post-procedure

1. Record any Adverse Experiences and Review subject diary for adverse experiences and dosing compliance.
2. Record changes to concomitant medications.
3. Perform abbreviated physical examination.
4. Perform and record vital signs.
5. Perform and record oximetry.
6. Perform post-op survey (see separate document for study tool).
7. Review pill bottles for medication usage.

10.5 Visit 5- 3 months post-procedure

1. Record any Adverse Experiences and Review subject diary for adverse experiences and dosing compliance.
2. Record changes to concomitant medications.
3. Perform abbreviated physical examination.
4. Perform and record vital signs.
5. Perform and record oximetry.
6. Perform post-op survey (see separate document for study tool).
7. Review pill bottles for medication usage.

10.6 Visit 6- 1 year post-procedure

1. Record any Adverse Experiences and Review subject diary for adverse experiences and dosing compliance.
2. Record changes to concomitant medications.
3. Perform abbreviated physical examination.
4. Perform and record vital signs.
5. Perform and record oximetry.
6. Perform post-op survey (see separate document for study tool).

7. Review pill bottles for medication usage.

10.7 Early Withdrawal Visit

1. Record any Adverse Experiences and Review subject diary for adverse experiences and dosing compliance.
2. Record changes to concomitant medications.
3. Perform abbreviated physical examination.
4. Perform and record vital signs.
5. Perform and record oximetry.
6. Perform post-op survey (see separate document for study tool).
7. Review pill bottles for medication usage.

11 ADVERSE EXPERIENCE REPORTING AND DOCUMENTATION

11.1 Adverse Events

An adverse event (AE) is any untoward medical occurrence in a clinical investigation of a patient administered a pharmaceutical product and that does not necessarily have a causal relationship with the treatment. An AE is therefore any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the administration of an investigational product, whether or not related to that investigational product. An unexpected AE is one of a type not identified in nature, severity, or frequency in the current Investigator's Brochure or of greater severity or frequency than expected based on the information in the Investigator's Brochure.

The Investigator will probe, via discussion with the subject, for the occurrence of AEs during each subject visit and record the information in the site's source documents. Adverse events will be recorded in the patient CRF. Adverse events will be described by duration (start and stop dates and times), severity, outcome, treatment and relation to study drug, or if unrelated, the cause.

AE Severity

The guidelines shown in Table 1 below should be used to grade severity. It should be pointed out that the term "severe" is a measure of intensity and that a severe AE is not necessarily serious.

Table 1. AE Severity Grading

Severity (Toxicity Grade)	Description
Mild (1)	Transient or mild discomfort; no limitation in activity; no medical intervention or therapy required. The subject may be aware of the sign or symptom but tolerates it reasonably well.
Moderate (2)	Mild to moderate limitation in activity, no or minimal medical intervention/therapy required.
Severe (3)	Marked limitation in activity, medical intervention/therapy required, hospitalizations possible.
Life-threatening (4)	The subject is at risk of death due to the adverse experience as it occurred. This does not refer to an experience that hypothetically might have caused death if it were more severe.

AE Relationship to Study Drug

The relationship of an AE to the study drug should be assessed using the following the guidelines in Table 2.

Table 2. AE Relationship to Study Drug

Relationship to Drug	Comment
Definitely	Previously known toxicity of agent; or an event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is not explained by any other reasonable hypothesis.
Probably	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is unlikely to be explained by the known characteristics of the subject's clinical state or by other interventions.
Possibly	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to that suspected drug; but that could readily have been produced by a number of other factors.
Unrelated	An event that can be determined with certainty to have no relationship to the study drug.

11.2 Serious Adverse Experiences (SAE)

An SAE is defined as any AE occurring at any dose that results in any of the following outcomes:

- death
- a life-threatening adverse experience
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity
- a congenital anomaly/birth defect

Other important medical events may also be considered an SAE when, based on appropriate medical judgment, they jeopardize the subject or require intervention to prevent one of the outcomes listed.

11.2.1 Serious Adverse Experience Reporting

Study sites will document all SAEs that occur (whether or not related to study drug) per UCSF CHR Guidelines. The collection period for all SAEs will begin after informed consent is obtained and end after procedures for the final study visit have been completed.

In accordance with the standard operating procedures and policies of the local Institutional Review Board (IRB)/Independent Ethics Committee (IEC), the site investigator will report SAEs to the IRB/IEC.

11.3 Medical Monitoring

Contact the Pediatric Surgery team to report medical concerns or questions regarding safety - (415) 476-2538. While subjects are inpatient, nursing staff and pediatric surgery service doctors are in-house 24 hours per day, 7 days per week.

12 DISCONTINUATION AND REPLACEMENT OF SUBJECTS

12.1 Early Discontinuation of Study Drug

A subject may be discontinued from study treatment at any time if the subject, the investigator, or the Sponsor feels that it is not in the subject's best interest to continue. The following is a list of possible reasons for study treatment discontinuation:

- Subject withdrawal of consent (or assent)
- Subject is not compliant with study procedures
- Adverse event that in the opinion of the investigator would be in the best interest of the subject to discontinue study treatment
- Protocol violation requiring discontinuation of study treatment
- Lost to follow-up
- Positive pregnancy test (females)

If a subject is withdrawn from treatment due to an adverse event, the subject will be followed and treated by the Investigator until the abnormal parameter or symptom has resolved or stabilized.

All subjects who discontinue study treatment should come in for an early discontinuation visit as soon as possible and then should be encouraged to complete all remaining scheduled visits and procedures.

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals.

12.3 Withdrawal of Subjects from the Study

A subject may be withdrawn from the study at any time if the subject, the investigator, or the Sponsor feels that it is not in the subject's best interest to continue.

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents. As noted above, subjects who discontinue study treatment early should have an early discontinuation visit.

12.4 Replacement of Subjects

Subjects who withdraw from the study treatment will not be replaced.

Subjects who withdraw from the study will not be replaced.

13 PROTOCOL VIOLATIONS

A protocol violation occurs when the subject or investigator fails to adhere to significant protocol requirements affecting the inclusion, exclusion, subject safety and primary endpoint criteria. Protocol violations for this study include, but are not limited to, the following:

- Failure to meet inclusion/exclusion criteria
- Interference with randomization
- Non-compliance with study drug regimen

Failure to comply with Good Clinical Practice (GCP) guidelines will also result in a protocol violation. The investigator will determine if a protocol violation will result in withdrawal of a subject.

When a protocol violation occurs, it will be discussed with the investigator and a Protocol Violation Form detailing the violation will be generated. This form will be signed by a Sponsor representative and the Investigator. A copy of the form will be filed in the site's regulatory binder and in the Sponsor's files.

14 STATISTICAL METHODS AND CONSIDERATIONS

Prior to the analysis of the final study data, a detailed Statistical Analysis Plan (SAP) will be written describing all analyses that will be performed. The SAP will contain any modifications to the analysis plan described below.

14.1 Data Sets Analyzed

All eligible patients who are randomized into the study and receive at least the initial analgesic application (cryoanalgesia or epidural catheter placement) and undergo Nuss procedure will be included in the analysis.

14.2 Demographic and Baseline Characteristics

The following demographic variables at screening will be recorded: gender, age, race, height and weight, medical comorbidities, severity of pectus excavatum deformity.

14.3 Analysis of Primary Endpoint

Our primary outcome will be post-operative hospital length of stay, which will be analyzed using a two-tailed unpaired t test, with results considered statistically significant for $p< 0.05$.

14.4 Analysis of Secondary Endpoints

Secondary outcomes include post-operative pain scores, pain medication usage, and side effects. Pain scores will be analyzed with Mann-Whitney rank sum tests (to compare between groups at each time point) and Wilcoxson signed-rank test (to compare scores within groups over time), with results considered statistically significant for $p< 0.05$.. Pain medication usage between the two groups will be analyzed with two-tailed unpaired t test, again with results considered statistically significant for $p< 0.05$.

Adverse events will be tabulated by treatment group and will include the number of patients for whom the event occurred, the rate of occurrence, and the severity and relationship to study drug.

14.5 Sample Size and Randomization

We used the UCSF Clinical & Translational Science Institute web-based sample size calculator (http://www.sample-size.net/sample-size-means/) to determine study size. Based on previous Nuss procedures over the past 2 years at our institution, we determined the mean length of hospitalization was 7 days, with a standard deviation of 1.3 days. From anecdotal data from our prior experience, as well as from speaking with other surgeons utilizing cryoablation, we expect patients in the cryoanalgesia group to have approximately 2 days shorter hospital stay. With type 1 error rate of 0.05 and type II error rate of 0.1 (yielding 90% power), our study would need 18 patients total. Allowing for some dropout, we will plan to enroll 20 patients to the study.

15 DATA COLLECTION, RETENTION AND MONITORING

15.1 Data Collection Instruments

The Investigator will prepare and maintain adequate and accurate source documents designed to record all observations and other pertinent data for each subject treated with the study drug.

Study personnel at each site will enter data from source documents corresponding to a subject's visit into the protocol-specific electronic Case Report Form (eCRF) OR paper CRF when the information corresponding to that visit is available. Subjects will not be identified by name in the study database or on any study documents to be collected by the Sponsor (or designee), but will be identified by a subject number.

For eCRFs: If a correction is required for an eCRF, the time and date stamps track the person entering or updating eCRF data and creates an electronic audit trail. *For paper CRFs:* If a correction is made on a CRF, the study staff member will line through the incorrect data, write in the correct data and initial and date the change.

The Investigator is responsible for all information collected on subjects enrolled in this study. All data collected during the course of this study must be reviewed and verified for completeness and accuracy by the Investigator.

15.2 Data Management Procedures

The data will be entered into a validated database. The Data Management group will be responsible for data processing, in accordance with procedural documentation. Database lock will occur once quality assurance procedures have been completed.

All procedures for the handling and analysis of data will be conducted using good computing practices meeting FDA guidelines for the handling and analysis of data for clinical trials.

15.3 Data Quality Control and Reporting

After data have been entered into the study database, a system of computerized data validation checks will be implemented and applied to the database on a regular basis. The study database will be updated in accordance with the resolved queries. All changes to the study database will be documented.

15.4 Archival of Data

The database is safeguarded against unauthorized access by established security procedures; appropriate backup copies of the database and related software files will be maintained. Databases are backed up by the database administrator in conjunction with any updates or changes to the database.

At critical junctures of the protocol (e.g., production of interim reports and final reports), data for analysis is locked and cleaned per established procedures.

15.5 Availability and Retention of Investigational Records

The Investigator must make study data accessible to the monitor, other authorized representatives of the Sponsor (or designee), IRB/IEC, and Regulatory Agency (e.g., FDA) inspectors upon request. A file for each subject must be maintained that includes the signed Informed Consent, HIPAA Authorization and Assent Form and copies of all source documentation related to that subject. The Investigator must ensure the reliability and availability of source documents from which the information on the CRF was derived.

All study documents (patient files, signed informed consent forms, copies of CRFs, Study File Notebook, etc.) must be kept secured for a period of two years following marketing of the investigational product or for two years after centers have been notified that the IND has been discontinued. There may be other circumstances for which the Sponsor is required to maintain study records and, therefore, the Sponsor should be contacted prior to removing study records for any reason.

15.6 Monitoring

Monitoring visits will be conducted by representatives of the Sponsor according to the U.S. CFR Title 21 Parts 50, 56, and 312 and ICH Guidelines for GCP (E6). By signing this protocol, the Investigator grants permission to the Sponsor (or designee), and appropriate regulatory authorities to conduct on-site monitoring and/or auditing of all appropriate study documentation.

15.7 Subject Confidentiality

In order to maintain subject confidentiality, only subject number will identify all study subjects on CRFs and other documentation submitted to the Sponsor. Additional subject confidentiality issues (if applicable) are covered in the Clinical Study Agreement.

16 ADMINISTRATIVE, ETHICAL, REGULATORY CONSIDERATIONS

The study will be conducted according to the Declaration of Helsinki, Protection of Human Volunteers (21 CFR 50), Institutional Review Boards (21 CFR 56), and Obligations of Clinical Investigators (21 CFR 312).

To maintain confidentiality, all laboratory specimens, evaluation forms, reports and other records will be identified by a coded number and initials only. All study records will be kept in a locked file cabinet and code sheets linking a patient's name to a patient identification number will be stored separately in another locked file cabinet. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the FDA. The Investigator must also comply with all applicable privacy regulations (e.g., Health Insurance Portability and Accountability Act of 1996, EU Data Protection Directive 95/46/EC).

16.1 Protocol Amendments

Any amendment to the protocol will be written by the investigator. Protocol amendments cannot be implemented without prior written IRB/IEC approval except as necessary to eliminate immediate safety hazards to patients. A protocol amendment intended to eliminate an apparent immediate hazard to patients may be implemented immediately, provided the IRBs are notified within five working days.

16.2 Institutional Review Boards and Independent Ethics Committees

The protocol and consent form will be reviewed and approved by the IRB/IEC of each participating center prior to study initiation. Serious adverse experiences regardless of causality will be reported to the IRB/IEC in accordance with the standard operating procedures and policies of the IRB/IEC, and the Investigator will keep the IRB/IEC informed as to the progress of the study. The Investigator will obtain assurance of IRB/IEC compliance with regulations.

Any documents that the IRB/IEC may need to fulfill its responsibilities (such as protocol, protocol amendments, Investigator's Brochure, consent forms, information concerning patient recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB/IEC. The IRB/IECs written unconditional approval of the study protocol and the informed consent form will be in the possession of the Investigator before the study is initiated. This approval must refer to the study by exact protocol title and number and should identify the documents reviewed and the date of review.

Protocol and/or informed consent modifications or changes may not be initiated without prior written IRB/IEC approval except when necessary to eliminate immediate hazards to the patients or when the change(s) involves only logistical or administrative aspects of the study. Such modifications will be submitted to the IRB/IEC and written verification that the modification was submitted and subsequently approved should be obtained.

The IRB/IEC must be informed of revisions to other documents originally submitted for review; serious and/or unexpected adverse experiences occurring during the study in accordance with the standard operating procedures and policies of the IRB; new information that may affect adversely the safety of the patients of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

16.3 Informed Consent Form

Informed consent will be obtained in accordance with the Declaration of Helsinki, ICH GCP, US Code of Federal Regulations for Protection of Human Subjects (21 CFR 50.25[a,b], CFR 50.27, and CFR Part 56, Subpart A), the Health Insurance Portability and Accountability Act (HIPAA, if applicable), and local regulations.

The Investigator will prepare the informed consent form, assent and HIPAA authorization and provide the documents to the Sponsor or designee for approval prior to submission to the IRB/IEC. The consent form generated by the Investigator must be acceptable to the Sponsor and be approved by the IRB/IEC. The written consent document will embody the elements of informed consent as described in the International Conference on Harmonisation and will also comply with local regulations. The Investigator will send an IRB/IEC-approved copy of the Informed Consent Form to the Sponsor (or designee) for the study file.

A properly executed, written, informed consent will be obtained from each subject prior to entering the subject into the trial. Information should be given in both oral and written form and subjects (or their legal representatives) must be given ample opportunity to inquire about details of the study. If appropriate and required by the local IRB/IEC, assent from the subject will also be obtained. If a subject is unable to sign the informed consent form (ICF) and the HIPAA authorization, a legal representative may sign for the subject. A copy of the signed consent form (and assent) will be given to the subject or legal representative of the subject and the original will be maintained with the subject's records.

16.4 Publications

The preparation and submittal for publication of manuscripts containing the study results shall be in accordance with a process determined by mutual written agreement among the study Sponsor and participating institutions. The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

16.5 Investigator Responsibilities

By signing the Agreement of Investigator form, the Investigator agrees to:

1. Conduct the study in accordance with the protocol and only make changes after notifying the Sponsor (or designee), except when to protect the safety, rights or welfare of subjects.
2. Personally conduct or supervise the study (or investigation).
3. Ensure that the requirements relating to obtaining informed consent and IRB review and approval meet federal guidelines, as stated in § 21 CFR, parts 50 and 56.
4. Report to the Sponsor or designee any AEs that occur in the course of the study, in accordance with §21 CFR 312.64.
5. Ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.
6. Maintain adequate and accurate records in accordance with §21 CFR 312.62 and to make those records available for inspection with the Sponsor (or designee).
7. Ensure that an IRB that complies with the requirements of §21 CFR part 56 will be responsible for initial and continuing review and approval of the clinical study.
8. Promptly report to the IRB and the Sponsor (or designee) all changes in the research activity and all unanticipated problems involving risks to subjects or others (to include amendments and IND safety reports).
9. Seek IRB approval before any changes are made in the research study, except when necessary to eliminate hazards to the patients/subjects.
10. Comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements listed in § 21 CFR part 312.

APPENDIX 1. SCHEDULE OF STUDY VISITS

	VISIT 1 pre-op	VISIT 2 procedure/hospitalization	VISIT 3 2 weeks post-op	VISIT 4 1 MONTH POST-OP	VISIT 5 3 MONTHS POST-OP	VISIT 6 1 YEAR POST-OP
Informed Consent	X					
Medical History	X					
Complete Physical Exam	X					
Abbreviated Physical Exam		X	X	X	X	X
Height	X	X				
Weight	X	X				
Vital Signs	X	X	X	X	X	X
Oximetry	X	X	X	X	X	X
Randomization	X					
Urine Pregnancy Test (females)		X				
Administration of Initial Analgesia (cryo vs. epidural)		X				
Survey Administration		X	X	X	X	X
Documentation of Pain Medication Usage		X	X	X	X	X
Counting of Returned Post-op Pain Medication			X	X	X	X
Initiate Subject Diary		X				
Subject Diary Review			X	X	X	X
Concomitant Medication Review	X	X	X	X	X	X
Adverse Experiences		X	X	X	X	X