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PROTOCOL TITLE: Contrast Enhanced Ultrasound for The Evaluation of Intraoperative Spinal Cord Injury Following Surgical Decompression of a Chronically Compressed Spinal Cord

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1.0 Objectives / Specific Aims

Aim 1: Evaluate and quantify through Contrast Enhanced Ultrasound (CEUS) micro- and macrovascular changes that lead to intraoperative areas of hypo and hyper-perfusion in the spinal cord of subjects that undergo elective cervicothoracic posterior decompression for chronic spinal cord compression. Question: Can CEUS identify micro- and macrovascular changes intraoperatively for posterior decompression of the cervicothoracic spine? In this aim, a hand-held intraoperative ultrasound probe will be used to collect sagittal and axial images of the spinal cord centered at the area of decompression in subjects that undergo posterior decompression of the cervical or thoracic spine. A bolus IV injection of DEFINITY® contrast agent (10 µL/kg DEFINITY®) will be given and continuous imaging will be obtained to record contrast inflow and washout before and after decompression to assess macro- and micro-vasculature. *We hypothesize that following decompression of a chronically compressed cervico-thoracic spinal cord, subjects will have detectable levels of microvascular changes causing areas of hyper- and hypoperfusion and reperfusion injury.*

Aim 2: Evaluate the efficacy of CEUS in detecting microvascular changes that correlate with Intraoperative Neuromonitoring (IONM) changes and predicting degree and recovery of post-operative neurologic deficits from intraoperative spinal cord injury. Question: Can micro- and macrovascular changes seen on CEUS be correlated with IONM changes? Additionally, can these changes be used to predict post-operative neurologic deficit and resolution in subjects undergoing elective and emergent cervicothoracic posterior decompression? A hand-held intraoperative ultrasound probe will be used to collect sagittal images of the spinal cord centered at the area of decompression in subjects that undergo posterior decompression of the cervical or thoracic spine. A bolus IV injection of DEFINITY® contrast agent (10 µL/kg DEFINITY®) will be given and continuous imaging will be obtained to record contrast inflow and washout before and after decompression to assess macro- and micro-vasculature. These finding will be correlated with changes seen on intraoperative neuromonitoring, postoperative imaging, as well as neurologic exam findings postoperatively. *We hypothesize that CEUS will detect micro-perfusion changes that are not perceptible on MRI and correlate with IONM changes that can predict both the degree of postoperative neurologic deficit and rate of recovery.*

2.0 Background

New neurologic deficits following posterior cervicothoracic decompression for chronic cord compression occur in 8.5% of patients and leads to both significant impact on quality of life as well as resource utilization[1–3]. There are about 1.6 million instrumented spinal procedures done in the United States each year, and a significant percentage of these include posterior decompressions. As our population continues to age, the number of spinal procedures will increase and the prevalence of these deficits will likely increase as well. The most commonly seen neurologic deficit is C5 palsy, which manifests with unilateral deltoid and biceps brachii weakness. While the majority of these patients have a resolution in these symptoms, there is an overall increased cost of $1,918 per patient that suffer from this complication[3–5]. Furthermore, due to the complex anatomy of the thoracic spine, surgery in this part of the spine leads to a similar rate of post-operative neurologic deficits. The impact of these complications at both the population and individual level has led to many investigations on the underlying cause of this type of injury to the spinal cord.

The etiology of these post-operative deficits can be identified when there is obvious iatrogenic injury to the cord from manual manipulation of the spinal cord and nerve roots. However, it is not uncommon to have post-operative neurologic deficits despite adequate intraoperative spinal cord decompression and no clear spinal cord injury visualized on postoperative imaging. In these cases, the underlying etiology is

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uncertain and has been hypothesized to be a result of ischemic injury, reperfusion injury or posterior migration of the cord leading to nerve root traction and injury. There is conflicting literature regarding each of these hypotheses and its possible that it may be multifactorial[2,3,6]. IONM is frequently used in spine surgery to evaluate for spinal cord injury; however, due to a lack of clarity, spine surgeons are currently seeking further intraoperative biomarkers to assess for unknown, reversible spinal cord injury that occurs intraoperatively.

Contrast enhanced ultrasound (CEUS) shows great promise for various neurosurgical applications as there is a paucity in intraoperative imaging techniques that can adequately depict the dynamic vascular and structural changes that the nervous system undergoes following surgery. CEUS has been shown to be safe and efficacious in the fields of cardiology and hepatology to identify microvasculature and perfusion changes and is now being explored in several fields including spine surgery[7–9]. CEUS provides real-time imaging of low-velocity blood flow in the microcirculation as well as higher-velocity blood flow in the larger vasculature and has been thoroughly studied in animal models to assess spinal cord injury and identify areas of macro- and microvascular changes, thus reinforcing its efficacy as a biomarker to evaluate spinal cord injury in humans[10–13].

To date, CEUS has not been investigated in patients suffering from chronic spinal cord compression that undergo posterior decompression, despite a reported rate of 8.5% of patients experiencing post-operative neurologic complications that are likely secondary to spinal cord injury[3]. There is a growing incidence and prevalence of both elective and emergent cervicothoracic spinal decompression as our population continues to age. Additionally, there is an already increasing use of non-invasive intra-operative ultrasound for spinal surgery[7]. Undoubtedly, the utilization of CEUS for evaluation of vascular changes during these surgeries and utility as a biomarker to identify spinal cord injury must be evaluated as a possible adjunct to already established intra-operative neuromonitoring techniques. Consequently, this will allow us to better understand the pathophysiology behind this subset of spinal cord injury and possibly establish mechanisms to reduce the incidence.

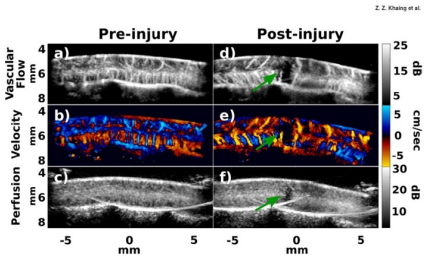
3.0 Intervention to be studied

Overview
This study will perform the following research specific procedures

1) Injection of DEFINITY® contrast agent
2) Intraoperative Contrast Enhanced Ultrasound use

Definitions and nomenclature
Contrast enhanced ultrasound (CEUS) is a readily used imaging modality that utilizes the injection of a microbubble contrast medium into the venous system to allow for improved ultrasound visualization of the micro- and macrovascular network of soft tissue structures. While CEUS, with the use of various microbubble contrast agents including DEFINITY contrast agent, is readily used and FDA approved for evaluation of the hepatic and cardiac systems, its role in spinal cord injury has only recently undergone investigation[9]. Khaing et al. demonstrated the efficacy of CEUS for monitoring spinal vascular structure and function in real time in a rodent spinal cord injury model[13]. This study demonstrates that this imaging modality can effectively assess spinal vasculature and blood flow in the macro- and microcirculation as well as identify areas of vascular changes following spinal cord injury (Figure 1).

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Furthermore, there is an IRB approved clinical trial ongoing (Clinical Trial Identifier: NCT04056988) that is currently investigating the efficacy of CEUS with the use of DEFINITY contrast as a biomarker in traumatic spinal cord injury and is currently enrolling human subjects. This ongoing clinical trial, as well as published safe dosing of DEFINITY contrast agent, has been reviewed and our protocol reflects these known dosing strategies[8,14]. The only contraindication to the use of DEFINITY contrast is patients with known or suspected hypersensitivity to perflutren lipid microsphere or its components, such as polyethylene glycol (PEG).
CEUS is a validated imaging modality that can provide real time information regarding the macro- and microvasculature of the spinal cord. As intraoperative ultrasound is already FDA approved and commonly used to assess the gross anatomical structure of the spinal cord following decompression, the use of a safe contrast agent to further improve its diagnostic efficacy must be evaluated. As DEFINITY contrast is already commonly used in a similar patient population to the one we are assessing, we believe that CEUS with the use of DEFINITY contrast agent, is safe and exposes potential participants to little harm.

Procedure Steps
The standard of care for cervicothoracic spinal cord compression causing radiculopathy and/or myelopathy involves a surgical posterior decompression where the lamina and ligamentum flavum are surgically removed to provide indirect decompression of the spinal cord. For subjects participating in this research, all surgical procedures will be the same, with the exception of the research-dedicated steps: 1) injection of DEFINITY contrast agent, 2) Intraoperative ultrasound use. For step 1, a 10 µL/kg bolus of DEFINITY® contrast agent will be injected intravenously and followed by a 10-cc injection of normal saline in order to assure complete injection of the contrast agent. Directly after injection, step 2 will commence where the ultrasound probe will be visualizing the spinal cord in the midline, sagittal plane. The ultrasound will remain visualizing the spinal cord for a total of 90 seconds until the contrast medium has dissipated from the operative field. Step 1 and 2 will be repeated at two separate portions of the case. First, it will occur at initial exposure before any decompression is performed to serve as a control. The second occurrence will be after decompression is complete but prior to wound closure.

Posterior cervicothoracic decompression surgery is reimbursed by insurers using a Diagnosis Related Group (DRG) model. This means that once a subject is preauthorized by an insurer to undergo this surgery, the surgery, hospital stay and all related procedures are bundled together and covered at a fixed cost. Costs related to research activity during this study (15 minutes total) will be charged to a research account and will not result in additional charges to the subject, hospital or the subject's insurer.
In this study, we will allow 15 minutes total to include setup and breakdown of the study-related equipment.

4.0 Inclusion and Exclusion Criteria/ Study Population

Only subjects considered an acceptable surgical candidate for posterior cervicothoracic decompression will be eligible for recruitment into this study. All diagnostic procedures, including imaging, will have

taken place prior to recruitment as part of the routine diagnosis and care of spine disorder patients. No subject will receive a CT or MRI scan in order to be included in this study, and subjects who did not have a CT or MRI as part of their clinical assessment will not be eligible for this study. Subject eligibility for this study will be based on the following inclusion/exclusion criteria:

Inclusion criteria:

- Subjects consented and scheduled for posterior cervicothoracic decompression surgery with the PI or Co-PI for the diagnosis of cervicothoracic myeloradiculopathy
- Age 18-80

Exclusion criteria:

- Pregnancy
- Pre-existing cardiac or pulmonary condition that would preclude study participation as determined by the anesthesiologist
- Subjects with known hypersensitivity to ultrasound contrast
- Subjects with known hypersensitivity to perflutren lipid microsphere or its components, such as polyethylene glycol (PEG)
- Subjects unable to actively participate in the consent process physically and/or cognitively
- Prior decompression at the intended level of decompression

The rationale for these criteria is as follows: The major risks to subjects in this study consist of hypersensitivity reaction to the contrast medium. Therefore, each eligible participant will undergo a medical review for any prior history of hypersensitivity to ultrasound contrast or its components.

5.0 Number of Subjects

The primary outcome of this study is to determine whether there are ultrasound detectable spinal cord perfusion changes following surgical decompression of a chronically compressed spinal cord. This study methodology is designed to address this primary outcome through an internal control design. The contrast enhanced echogenicity of the spinal cord will be collected prior to decompression which will serve as our control. Following decompression, the contrast enhanced echogenicity of the same spinal cord area will again be assessed and compared to that of the control. A change in spinal cord perfusion is defined as a 10% change (reduction or elevation) in ultrasonographic echogenicity measured in dB/MHz/cm. Thus, there are three possible outcomes following decompression for each subject; similar perfusion, hypoperfusion, or hyperperfusion. Therefore, for this primary outcome, a one group chi-square test with a 0.050 two-sided significance level will have 80% power to detect the difference between the Null hypothesis proportion, p0, of 0.330 and the alternative proportion, pA, of 0.630 when the sample size is 20.

Additionally, the secondary outcome of this study is correlating the results of the CEUS findings to functional outcomes of subjects following surgery, which will be recorded by Frankel scoring. Frankel score is a validated scale to assess the functional outcome of patients suffering from spinal cord injury.[15] For this secondary outcome, a two group t-test with a 0.050 two-sided significance level will have 80% power to detect the difference between a Group 1 Frankel score mean of 2.000 and a Group 2 Frankel score mean of 1.000, a difference in means of 1.000, assuming that the common standard deviation is 0.500, when the sample sizes in the two groups are 6 and 6, respectively (a total sample size of 12).

Given the power analysis performed, 20 subjects will provide sufficient power to assess our primary and secondary outcomes. The Co-investigators in this study performs approximately 200 posterior

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cervicothoracic surgeries per year, thus providing confidence that this study will be able to meet recruitment goal within one year.

6.0 Setting

The data for this study will be recorded in the operating room at MUSC. Coded and identifiable data will be stored electronically on a secure server in separate folders. Signed consent forms will be located in a locked cabinet in a locked laboratory in the Clinical Sciences Building at MUSC.

7.0 Recruitment Methods

An eligibility screening checklist will be used prior to completing the informed consent process. The screening will not require access to PHI. The screening checklist will contain only basic inclusion and exclusion criteria for the study. The purpose of the screening is to determine whether the potential subject meets general study eligibility prior to executing the informed consent process. This process will work in the following manner: During a preoperative clinic visit with the PI or Co-I the PI/Co-I will ask the subject if they would like to learn more about the study. If so, the PI/Co-I will then complete the screening checklist then sign and date the form. If the subject meets general study eligibility, the PI/Co-I will review the protocol and consent form with the subject and then leave the room in order to give the subject sufficient time to read the consent form.

The PI/Co-I are the surgeons performing the surgery, and will therefore have the knowledge to carefully explain the research study. Careful attention will be placed on good communication. The PI/Co-I will be sure to emphasize that participation in the study is not necessary for the standard of care surgery. He will also emphasize that the subject can withdraw from the study at any time prior to the surgery.

8.0 Consent Process

Once the subject has been given an opportunity to review the consent form in detail, the PI and/or Co-Is, along with another member of the IRB-approved personnel team for this study, will re-enter the room and go over the sections of the consent with the participant. The Co-I will also review the HIPAA authorization statement embedded in the consent. They will answer any questions the subject may have. The voluntary nature of the study will again be emphasized. It will also be emphasized that the subject may exercise their right to take the consent form home to discuss with family and friends prior to signing. For subjects that provide consent to the research study during their clinic visit, a copy of the signed and dated consent form will be provided to them before leaving the clinic. The subject will be given a copy of this form. Original forms will be placed in a locked cabinet in a locked laboratory in the Clinical Sciences Building at MUSC. On the morning of surgery, IRB-approved study personnel will once again review the study procedures and study consent document – emphasizing the voluntary nature of the study and the ability of the patient to withdraw at any time. Subjects who did not sign the consent and HIPAA authorization forms during their clinic visit and who still wish to participate in the study will be able to sign the forms on the morning of surgery, at which time a copy of the signed and dated forms will be provided to them.

Subjects will be informed during the consent process that information about them (including identifiable private information) may have all of their identifiers removed and used for future research studies or distributed to other researchers for future research without additional informed consent from them.

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9.0 Study Design / Methods

The study will begin with a medical chart review by the research staff, who will extract data elements from the subject's electronic medical record (EMR) to be stored in a database (see Data Management section). For female patients of childbearing age, the medical record will be reviewed for pre-operative pregnancy test to confirm that the subject is not pregnant prior to enrollment. If there is no pregnancy test present, the subject will undergo a pregnancy test to confirm that they are not pregnant. If this pregnancy test is found to be positive, the subject will be removed from the study. All subjects will have a chart review to evaluate for any pre-existing cardiac or pulmonary conditions that would meet exclusion criteria. Additionally, all subjects will undergo a pre-anesthesia evaluation by a board-certified anesthesiologist as per standard of care for this procedure, which includes a review of medical history, a thorough physical exam, an EKG, and review of vitamin and prescription medication use. This occurs 1-week prior to the procedure. The results of this evaluation will be reviewed for the development of any cardiac or pulmonary conditions that would exacerbate the risks for the enrolled subject. Should any pre-existing cardiac or pulmonary condition be identified in the chart review, the patient will be excluded.

Once in the operating room and prior to the surgical procedure commencing, a research "Time-Out" will be performed. This research "Time-Out" will call for the operating room to be silent while the PI or Co-I informs the operative staff and anesthesiologist that this subject will be undergoing the research steps as detailed below. The PI or Co-I will explain that there will be two separate injections of a contrast agent that has a reported risk of hypersensitivity reaction and cardio-pulmonary adverse reactions in a small subset of patients. The PI or Co-PI will confirm that the anesthesiologist present has access to the code cart and appropriate medications should the subject experience any adverse reaction. Lastly, the PI or Co-I will inform the anesthesiologist that they will be alerted once the contrast is injected.

Following this, the study will implement the steps described below.

Step 1: CEUS Image Acquisition Prior To Decompression
Following standard posterior, subperiosteal dissection of the cervicothoracic spine, the interlaminar space of the compressed spinal segment of interest will be identified. The wound will be filled with sterile saline and the CEUS will be brought into the surgical field in a sterile manner. The CEUS hockey stick probe will be introduced into the wound, and the underlying spinal cord will be visualized between the interlaminar ligament in the axial plane. Once an adequate, pre-contrast image is obtained, we will begin the injection of the ultrasound contrast. Prior to injection, we will confirm hemodynamic stability with the anesthesia team. Once confirmed, a 10 µL/kg bolus of DEFINITY® contrast agent will be prepared and injected by the PI or Co-I into the intravenous catheter. This will be followed by a 10-cc injection of normal saline in order to assure complete injection of the contrast agent. The ultrasound probe will remain visualizing the spinal cord for a total of 90 seconds. Following 90 seconds of image acquisition, Step 1 will be completed.

Step 2: CEUS Image Acquisition After Decompression
Following standard, posterior laminectomy and resection of ligamentum flavum, the area of compression and underlying spinal cord will be identified. The wound will be filled with sterile saline and the CEUS will be brought into the surgical field in a sterile manner. The CEUS hockey stick probe will be introduced into the wound, and the underlying spinal cord will be visualized in the axial and sagittal plane, with the probe directed towards the area of significant compression and interest. Once an adequate, pre-contrast image is obtained, we will begin the injection of the ultrasound contrast. Prior to injection, we will confirm hemodynamic stability with the anesthesia team. Once confirmed, a 10 µL/kg bolus of

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DEFINITY® contrast agent will be prepared and injected by the PI or Co-I into the intravenous catheter. This will be followed by a 10-cc injection of normal saline in order to assure complete injection of the contrast agent. The ultrasound probe will remain visualizing the spinal cord for a total of 90 seconds. Following 90 seconds of image acquisition, Step 2 will be completed.

It is important to note that the ultrasound probe will not penetrate the skin, dura, or spinal cord at any time. The ultrasound probe will remain outside the spinal cord itself, and will acquire images through the sterile saline medium.

Subject Follow-up
Following closure of the wound, the procedure will be complete. Research staff will follow up with each participant by phone within a week of the procedure to make sure the participants are not having any adverse reactions from the contrast administered. Additionally, research staff will review each participant's medical record at 1, 6, and 12 months after the procedure and the PI's will assess the subject in the neurosurgical clinic as visits at these time points are considered standard of care within our department. Clinical data will include a physical exam, neurological exam and assessment for any adverse reactions to the contrast provided. Additionally, any additional treatments, tests or procedures the participant may have had in the postoperative period will be reviewed and recorded.

10.0 Data Management

Once a subject is screened, the results of their screen will be recorded. We will maintain a database that accurately reflects all the subjects that were screened and the results of eligibility evaluation (screen failure vs enrolled). For enrolled subjects, the specific types of data that will be collected include: 1) clinical data including demographics, clinical data, radiographic data, and past medical history 2) intraoperative and post-operative clinical data including intraoperative neuromonitoring physiology data and 3) ultrasound imaging data (dicom files). Raw data will remain on data acquisition machines with encrypted hard drives and stored behind a locked door when not directly supervised in the operating room. Data will be copied from all data acquisition machines to a password protected network server under the participant ID. This will constitute the master copy of all data for the study and will consist of separately stored coded and identifiable folders. The purpose of keeping a copy of the raw data on the data acquisition machines is in case the server is interrupted and it is necessary to reconstruct the data.

A linking database associating the participant ID and subject identifiers will be maintained on MUSC Box, separately from the research data. Subject identifiers to be collected include first and last name, medical record number, gender, and telephone numbers. Telephone and medical record numbers are required for the 7-day phone call and post-operative chart reviews. Findings from the phone call and chart review will be stored in the linking database. Additionally, we will collect information pertaining to disease severity for each subject, including year of diagnosis, symptomology, location of surgery, and pre- and post-surgical pain and motor scales. These measures will assist in correlating our collected ultrasound data with change in symptoms post-operatively.

Finally, radiographic imaging data pre- and post-operatively is used to plan the spine surgery and assess degree of decompression for each subject. We will save this data on a password protected network server in order to analyze and correlate with our intraoperative ultrasound imaging data. Only IRB-approved personnel will have access to the linking database. Consent forms will be stored in a locked cabinet in a locked laboratory in the Clinical Sciences Building at MUSC.

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11.0 Provisions to Monitor the Data to Ensure the Safety of Subjects

Data and Safety Monitoring Plan

The proposed research involves use of an off-label contrast agent and warrants oversight by a Data Safety Monitoring Board (DSMB). The primary purpose of the DSMB is to ensure the safety of participants and the validity and integrity of data collected during the study. The overall framework involves biannual review of the enrollment, safety and adverse event data, and quality control data by the DSMB throughout the period of the proposed research.

DSMB composition

The DSMB will be appointed by the PI and will be composed of individuals who are not on the study team and have the following qualifications: (1) a board-certified neurosurgeon who also is a spinal cord injury researcher and is experienced in care of patients with spine disorders, (2) a biostatistician with expertise in design and analysis of clinical trials and (3) a clinical ultrasonographer with expertise in contrast enhanced ultrasound. A chair will be selected amongst the three board members and will be responsible for communicating with the IRB. These three individuals bring substantial expertise adequate to monitor data and safety for the proposed research.

DSMB responsibilities

The responsibilities of the DSMB are as follows. Prior to any enrollment, the DSMB will review the study design, protocol, recruitment/enrollment plan, statistical analysis plan, and data and safety monitoring plan. Once enrollment begins, the DSMB will convene every 6 months to review the enrollment data, the safety and adverse event data and quality control data (see below for detailed data description). The DSMB will review the aggregated summary data as well as the individual participants' data (de-identified). The DSMB may provide recommendations for any safety concerns. The DSMB may recommend stopping the study early if the protocol has significant safety concerns. The DSMB will review the results and document their reviews in writing. In summary, the stopping rules are if the study has unanticipated safety concerns. The DSMB will also be able to make recommendations for appropriate action to maintain a reasonable safety profile for the study. The chair of the DSMB will write a report for the IRB to review that summarizes oversight activities, DSMB recommendations and any concerns regarding participant safety. The written letter will be given to the PI who will subsequently submit to the IRB for review.

Safety reporting to the DSMB

Subjects may report safety issues with the study directly to the PI, any member of the PI's clinical and/or research teams, the MUSC Family Care Liaison, the IRB and/or ORI Director. In the absence of any such reports, the PI will systematically attempt to uncover any safety issues experienced by a subject during the one-week follow-up phone call and/or 30-day chart review. Any of the following will be reported to the DSMB if noted by the subject during the one-week follow-up phone call: any hypersensitivity reaction symptoms (rash, itchiness, flushing, difficulty breathing, etc.) and/or return to the hospital. Any of the following will be reported to the DSMB if noted during the 30-day chart review: return to hospital, evaluation by another provider for findings related to spine surgery, imaging, lab values and/or other reports indicating complications from spine surgery or contrast administration. All the above-mentioned issues are outside of what would be reasonably expected for a normal postoperative course following spine surgery. In accordance with HRPP Section 4.7, all the above-mentioned issues constitute adverse events and will be submitted to the DSMB for review (at 6 month intervals) in addition to being recorded in the adverse events log. Events that are unexpected, related or possibly related to a subject's participation in the research and suggests that the research places subjects or others at greater risk of harm

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than was previously known or recognized constitute Unanticipated Problems Involving Risks to Subjects or Others (UPIRSOs) and must be reported directly to the IRB (in addition to the DSMB). Finally, all deaths will be reported to the IRB within 24 hours of learning of the death, unless the death is expected (e.g., due to disease progression).

12.0 Withdrawal of Subjects (if applicable)

Subjects may be withdrawn from the study without their consent, including stopping participation for safety reasons. For example, if during operation it becomes evident that continuing with the study protocol would present risk in excess of that outlined in this document, data collection for that subject may be terminated even if the planned surgical procedure is continued. These conditions may include hypersensitivity reaction or inability to acquire adequate images due to anatomical constraints. At any time, subjects may also express desire to withdraw from the study. We will instruct the subjects that, to withdraw from the study, all they need to do is to contact one of the members of the study team and express the desire to be excluded from the study.

Subjects may be terminated or withdrawn from the study for the following reasons:

- Voluntary withdrawal – meaning that subject voluntarily chooses not to further participate in the study
- Subjects may be withdrawn from the study without their consent, including stopping participation for safety reasons.

NOTE: For those subjects considered lost to follow-up (see definition above), the site will, at a minimum, make a concerted effort to confirm that the subject is not deceased (e.g., active search of death indices will be performed to ensure the subject remains alive). The site will also document at least 3 attempts to contact the subject by telephone.

13.0 Risks to Subjects

Intraoperative Ultrasound

The use of intraoperative ultrasound to evaluate soft tissue structures including the spinal cord and underlying compressive pathology during posterior cervicothoracic decompression is FDA approved and frequently used amongst spine surgeons[7,16,17]. As the imaging quality has increased and the transducer size has decreased while maintain effective image quality, intraoperative ultrasound has become ubiquitous in the neurosurgical operating room. As images are acquired through low frequency sound waves, without direct contact with tissue of interest, and lacking any ionizing radiation, intraoperative ultrasound is a safe imaging modality that is considered standard of care within our department. The use of intraoperative ultrasound for spinal cord decompression has been associated with decreased radiation exposure, safer dissection, and decreased operative time as it allows for direct visualization of neural structures and compressive pathology[18].

Microbubble Contrast Administration

In a prospective, multicenter, open-label registry of 1053 patients receiving DEFINITY in routine clinical practice, heart rate, respiratory rate, and pulse oximetry were monitored for 30 minutes after DEFINITY administration. No deaths or serious adverse reactions were reported, suggesting that these reactions are

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unlikely to occur at a rate of more than 0.3% when DEFINITY is used. A total of 1716 patients were evaluated in pre-market clinical trials of activated DEFINITY, and reported rates of adverse reactions included injection site reactions (.6%), dizziness (.6%), nausea (1%), flushing (1.1%). Additionally, among the 1716 study patients, 19 (1.1%) suffered serious cardiopulmonary adverse reactions. However, because pre-market clinical trials are conducted under widely varying conditions, these rates may not reflect the rates observed in practice[8,9,14]. A table with the various adverse reaction encountered in this clinical trial and reported in the package insert can be seen in the table below. Other adverse reactions that occurred in ≤0.5% of the activated DEFINITY-dosed patients were:

Body as a Whole: Fatigue, fever, hot flushes, pain, rigors, and syncope
Cardiovascular: Abnormal ECGs, bradycardia, tachycardia, palpitation, hypertension and hypotension
Digestive: Dyspepsia, dry mouth, tongue swelling, toothache, abdominal pain, diarrhea and vomiting
Hematology: Granulocytosis, leukocytosis, leukopenia, and eosinophilia
Musculoskeletal: Arthralgia
Nervous System: Leg cramps, hypertonia, vertigo and paresthesia
Platelet, Bleeding, and Clotting: Hematoma
Respiratory: Coughing, hypoxia, pharyngitis, rhinitis and dyspnea
Special Senses: Decreased hearing, conjunctivitis, abnormal vision and taste perversion
Skin: Pruritus, rash, erythematous rash, urticaria, increased sweating, and dry skin
Urinary: Albuminuria

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Table 1 New-Onset Adverse Reactions Occurring in ≥0.5% of All DEFINITY-Treated Subjects

	DEFINITY (N=1716)	
Total Number of Adverse Reactions	269	
Total Number of Subjects with an Adverse Reaction	144	(8.4%)
Body system Preferred term	n	(%)
Application Site Disorders	11	(0.6)
Injection Site Reactions	11	(0.6)
Body as a Whole	41	(2.4)
Back/renal pain	20	(1.2)
Chest pain	13	(0.8)
Central and peripheral nervous system disorder	54	(3.1)
Headache	40	(2.3)
Dizziness	11	(0.6)
Gastrointestinal system	31	(1.8)
Nausea	17	(1.0)
Vascular (extracardiac) disorders	19	(1.1)
Flushing	19	(1.1)

N=Sample size 1716 subjects who received activated DEFINITY
n=Number of subjects reporting at least one Adverse Reaction

All subjects will be under general anesthesia with a board certified anesthesia provider present at all times, as is standard for this surgery. They will have continuous vital sign monitoring using standard monitors and should a hypersensitivity reaction be suspected; anesthesia personnel will be able to readily recognize and treat it. Therefore, in the unlikely case that there is a hypersensitivity reaction leading to hypotension or airway edema, the subject's hypotension will immediately be identified and treated.

While the use of this dosing strategy to evaluate spinal cord injury is a novel technique, it has been used and published in a similar patient population and has demonstrated both efficacy and safety. Two series have been published using the same dosing strategy proposed in this protocol for patients with intramedullary spinal tumors.[19,20] The patients included in these two series included patients with spinal cord pathology undergoing elective spinal cord surgery, which is similar to the proposed population of this protocol. No adverse effects to the contrast agents were identified or reported in either of these series, while also demonstrating effective visualization of both the intrinsic pathology and the associated spinal cord perfusion.

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Loss of Confidentiality

Additional risks include loss of confidentiality. To mitigate this risk, a linking database associating the participant ID and subject identifiers will be maintained separately from the research data on a password protected server and only IRB-approved personnel will have access to the linking database.

14.0 Potential Benefits to Subjects or Others

This research is not expected to provide direct benefit to individual subjects but may lead to improvements in care for patients suffering from spinal cord injury in the future. A successful study outcome may contribute to this improvement and may be used in future patients undergoing similar procedures.

15.0 Sharing of Results with Subjects

Due to the extensive amount of time needed for data processing, it will not be possible to disclose individual study results to subjects or their healthcare providers. Incidental findings and other results of diagnostic tests associated with routine care of subjects will be shared with the subject and their physician by phone, writing and/or electronic medical record communication by the PI.

16.0 Drugs or Devices (if applicable)

The ultrasound contrast will be stored with the MUSC IDS pharmacy in a designated, refrigerated area. On the day of data collection, the DEFINITY ultrasound contrast will be transported to the operating room at MUSC then immediately transported back to the MUSC IDS pharmacy at the conclusion of the operation. The DEFINITY ultrasound contrast will be transported to the operating room in a designated and labeled cooler.

References

1. Sakaura H, Hosono N, Mukai Y, Ishii T, Yoshikawa H. C5 Palsy after Decompression Surgery for Cervical Myelopathy: Review of the Literature. *Spine*. 2003;28(21):2447-2451. doi:10.1097/01.BRS.0000090833.96168.3F

2. Badiee RK, Mayer R, Pennicooke B, Chou D, Mummaneni PV, Tan LA. Complications following posterior cervical decompression and fusion: a review of incidence, risk factors, and prevention strategies. *J Spine Surg*. 2020;6(1):323-333. doi:10.21037/jss.2019.11.01

3. Bydon M, Macki M, Kaloostian P, et al. Incidence and Prognostic Factors of C5 Palsy: A Clinical Study of 1001 Cases and Review of the Literature. *Neurosurgery*. 2014;74(6):595-605. doi:10.1227/NEU.0000000000000322

4. Wang T, Wang H, Liu S, Ding WY. Incidence of C5 nerve root palsy after cervical surgery. *Medicine (Baltimore)*. 2017;96(45):e8560. doi:10.1097/MD.0000000000008560

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5. Bydon M, Macki M, Aygun N, et al. Development of postoperative C5 palsy is associated with wider posterior decompressions: an analysis of 41 patients. *Spine J*. 2014;14(12):2861-2867. doi:10.1016/j.spinee.2014.03.040

6. Anand SK, Macki M. What have we learned from C5 palsy – A short communication. *J Clin Neurosci*. 2020;81:111-112. doi:10.1016/j.jocn.2020.09.044

7. Hwang BY, Mampre D, Ahmed AK, et al. Ultrasound in Traumatic Spinal Cord Injury: A Wide-Open Field. *Neurosurgery*. 2021;89(3):372-382. doi:10.1093/neuros/nyab177

8. Weiss RJ, Ahmad M, Villanueva F, et al. CaRES (Contrast Echocardiography Registry for Safety Surveillance): a prospective multicenter study to evaluate the safety of the ultrasound contrast agent definity in clinical practice. *J Am Soc Echocardiogr Off Publ Am Soc Echocardiogr*. 2012;25(7):790-795. doi:10.1016/j.echo.2012.04.002

9. Main ML. Contrast echocardiography: safety in numbers. *Cardiology*. 2012;122(4):248-250. doi:10.1159/000341246

10. Dubory A, Laemmel E, Badner A, et al. Contrast enhanced ultrasound imaging for assessment of spinal cord blood flow in experimental spinal cord injury. *J Vis Exp JoVE*. 2015;(99):e52536. doi:10.3791/52536

11. Soubeyrand M, Laemmel E, Dubory A, Vicaut E, Court C, Duranteau J. Real-time and spatial quantification using contrast-enhanced ultrasonography of spinal cord perfusion during experimental spinal cord injury. *Spine*. 2012;37(22):E1376-1382. doi:10.1097/BRS.0b013e318269790f

12. Huang L, Chen K, Chen FC, et al. Intraoperative contrast-enhanced ultrasonography for microcirculatory evaluation in rhesus monkey with spinal cord injury. *Oncotarget*. 2017;8(25):40756-40764. doi:10.18632/oncotarget.17252

13. Khaing ZZ, Cates LN, DeWees DM, et al. Contrast-enhanced ultrasound to visualize hemodynamic changes after rodent spinal cord injury. *J Neurosurg Spine*. 2018;29(3):306-313. doi:10.3171/2018.1.SPINE171202

14. Wei K, Main ML, Lang RM, et al. The effect of Definity on systemic and pulmonary hemodynamics in patients. *J Am Soc Echocardiogr Off Publ Am Soc Echocardiogr*. 2012;25(5):584-588. doi:10.1016/j.echo.2012.01.019

15. van Middendorp JJ, Goss B, Urquhart S, Atresh S, Williams RP, Schuetz M. Diagnosis and Prognosis of Traumatic Spinal Cord Injury. *Glob Spine J*. 2011;1(1):1-8. doi:10.1055/s-0031-1296049

16. Ganau M, Syrmos N, Martin AR, Jiang F, Fehlings MG. Intraoperative ultrasound in spine surgery: history, current applications, future developments. *Quant Imaging Med Surg*. 2018;8(3):261-267. doi:10.21037/qims.2018.04.02

17. Vasudeva VS, Abd-El-Barr M, Pompeu YA, Karhade A, Groff MW, Lu Y. Use of Intraoperative Ultrasound During Spinal Surgery. *Glob Spine J*. 2017;7(7):648-656. doi:10.1177/2192568217700100

18. Saway BF, Alshareef M, Lajthia O, et al. Ultrasonic spine surgery for every thoracic disc herniation: a 43-patient case series and technical note demonstrating safety and efficacy using a partial transpedicular thoracic discectomy with ultrasonic aspiration and ultrasound guidance. *J Neurosurg Spine*. Published online November 19, 2021:1-9. doi:10.3171/2021.8.SPINE21819

19. Han B, Wu D, Jia W, Lin S, Xu Y. Intraoperative Ultrasound and Contrast-Enhanced Ultrasound in Surgical Treatment of Intramedullary Spinal Tumors. *World Neurosurg*. 2020;137:e570-e576. doi:10.1016/j.wneu.2020.02.059

20. Barkley A, McGrath LB, Hofstetter CP. Intraoperative contrast-enhanced ultrasound for intramedullary spinal neoplasms: patient series. *J Neurosurg Case Lessons*. 2021;1(7). doi:10.3171/CASE2083

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