Markers of Alzheimers Disease and Cognitive Outcomes After Perioperative Care

NCT01993836

Document Date: December 16, 2019

CLINICAL STUDY PROTOCOL

Markers of Alzheimers Disease and Cognitive Outcomes after Perioperative Care.

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1. STUDY SYNOPSIS

PROJECT:

Alzheimer's disease, the most common type of dementia, is a rising public health issue in the United States and worldwide, with a prevalence rising to near 50% by age 85. Epidemiologic studies have linked anesthetic exposure to the development of dementia and Alzheimer's disease, as well as milder forms of cognitive decline (i.e. post operative cognitive decline, or POCD). Furthermore, animal studies have shown that inhaled anesthetic agents increase the levels of Alzheimer's disease related neural markers (such as amyloid beta and tau). However, no studies to date have examined whether inhaled anesthetics alter brain levels of these Alzheimer's associated markers in humans, and/or whether such changes (if they occur) correlate with POCD or other cognitive changes after surgery and anesthesia, or whether inhaled anesthetics cause changes in brain structure or connectivity. Further no studies to date have examined these issues in surgical patients and age matched non-surgical controls (ie community dwelling older adults).

Here, we propose to measure Alzheimer's disease related neural markers in the cerebrospinal fluid (CSF) from patients exposed to anesthesia during surgery, as well as in non surgical controls. Surgical patients in the study will be randomized to either receive inhalation anesthesia with isoflurane or intravenous anesthesia with propofol; non-surgical controls in this study will not receive any anesthesia or surgery. We will also record non-invasive electroencephalogram (EEG) data from the surgical patients during surgery, and during a short interval before and after anesthesia and surgery. CSF and blood samples will each be collected at the time of anesthesia induction +/- 1 hour and then 24 hours +/- 1 hour later, 6 weeks +/- 3 weeks later, and 1 year +/- 2 months later in surgical patients, and at the cognate time intervals in non-surgical controls. CSF samples will be collected via a lumbar puncture. Blood samples will be drawn from an intravenous or intra-arterial line or from a sterile venipuncture. CSF samples will be assayed for amyloid beta, tau, and other Alzheimer's disease-associated markers; blood samples will be assayed for serum inflammatory markers and used for genotyping studies. Study patients will also undergo magnetic resonance imaging (MRI/fMRI) scans before surgery and 6 weeks later, or at the cognate time points in non-surgical controls. These studies should clarify the effect of common anesthetic agents on Alzheimer's disease related neural markers.

STUDY TITLE:
CSF Markers of Alzheimers Disease and Cognitive Outcomes after Isoflurane versus Propofol Anesthesia

OBJECTIVE:
The primary objective is to assess the correlation between perioperative change in CSF Markers of Alzheimer's Disease and perioperative cognitive change. [Time Frame: up to 6 weeks]
We will assess the magnitude of the pearson correlation coefficient for the relationship between perioperative change in CSF Markers of Alzheimers Disease and the perioperative change in the continuous cognitive index score.

DESIGN:
Patients undergoing urologic or general surgical procedures will be prospectively enrolled, will undergo pre-operative cognitive testing and will be randomized to receive inhalation anesthesia with isoflurane or intravenous anesthesia with propofol. Additionally, non-surgical controls (community dwelling elderly individuals) will undergo the same study procedures at the cognate time intervals (with the exception of anesthesia and surgery.

CSF samples (10 ml each—approximately 2 teaspoons) will be obtained from a lumbar puncture at the beginning of the case +/-1 hour, and 24 hours +/- 1 hour later. Blood samples (10 ml each—approximately 2 teaspoons) will be obtained at these time points as well**.** Study patients will return to the hospital 6 weeks +/- 3 weeks and 1 year +/- 2 months later to undergo repeat cognitive testing,lumbar puncture to obtain CSF and blood draws. Non surgical controls will undergo these same procedures at the cognate time intervals.

In order to minimize bias, investigators conducting the laboratory assays on the CSF will be blinded to study group assignment (both anesthetic type, and surgical vs non surgical group) during the course of the study. CSF and blood samples will be collected by a trained care provider under sterile conditions. CSF specimens will be analyzed for amyloid beta, tau and other related Alzheimers-associated neural markers using previously established protocols as described below. Blood samples will be used for genotyping and metabolic/inflammatory marker assays as described below. Blood and CSF samples will be stored at -80 degrees Celcius. All assay measurements will be performed at Duke University Medical Center or in the accredited laboratory of a collaborator. Completed case report forms (CRFs) and CSF samples will be stored at Duke University Medical Center, CRFs will be locked in Dr. Mathew's or Dr. Berger's office or stored on Duke University Health System or Department of Anesthesiology computer servers within the Duke firewall, and subject samples will be held in freezers in the Department of Anesthesiology and/or Duke University Medical Research Facilities.

SUBJECTS:
100 patients presenting for surgical procedures at Duke, and 100 non-surgical controls (community dwelling older individuals). Up to 70 surgical patients and 70 non-surgical controls will undergo pre and post-operative MRI/fMRI scans. Only subjects who complete cognitive testing and blood/CSF sampling at all study time points will count towards the enrollment targets of 100 surgical patients and 100 non surgical controls. Surgical patients may participate in the study more than once if they desire, if they are having a second surgery after they have already completed all study procedures (including the 1 year time point). There are no added risks to subjects from participating in the study additional times, beyond the risks already discussed in the ICF.

STUDY START DATE:
June 15, 2013

STUDY DURATION:
36 months total, approximately 1 year duration for each patient.

2. STUDY OBJECTIVES

2.1. Primary Objective

The primary objective is to assess the correlation between perioperative change in CSF Markers of Alzheimers Disease and perioperative cognitive change.
[Time Frame: up to 6 weeks]
We will assess the magnitude of the pearson correlation coefficient for the relationship between perioperative change in CSF Markers of Alzheimers Disease and the perioperative change in the continuous cognitive index score.

3. Secondary Objectives
1.Continuous cognitive index score change [Time Frame: 6 weeks]
We will examine the difference in the continuous cognitive index score over time in the subjects treated with propofol versus those treated with isoflurane.
Assess the correlation between perioperative change in CSF Markers of Alzheimers Disease and perioperative cognitive change.

2. Assess CSF Markers of Alzheimers Disease [Time Frame: up to 6 weeks]
We will examine CSF Markers of Alzheimer's Disease over time in the subjects treated with propofol versus those treated with isoflurane.

3.Perioperative CSF Tau/Abeta ratio change [Time Frame: 24 hours]
We will measure the perioperative change in the CSF tau/Abeta ratio from the start of anesthesia/surgery to 24 hours later.

3. Exploratory Objectives

- Describe the relationship between anesthetic exposure and CSF biomarker changes, and demographics (age, gender, and race).

- Describe the relationship between CSF biomarker changes and serum markers of metabolic and/or inflammatory status.
- Describe the relationship between anesthetic exposure and biomarker changes and serum markers of metabolic and/or inflammatory status.
- Describe the relationship between anesthetic exposure and biomarker changes and neuroimaging results obtained as dictated by routine standard of care.
- Describe the relationship between anesthetic exposure and biomarker changes and depth of anesthesia, based on average BIS monitoring signal.
- Describe the relationship between anesthetic exposure and biomarker changes and the time of day that surgery is performed.
- Describe the relationship between anesthetic exposure and biomarker changes with genetic polymorphisms in ApoE and other genes related to inflammation, metabolism, brain function and/or Alzheimer's disease.
- Determine the relationship between anesthetic exposure type and MRI/fMRI imaging variables of interest.
- Determine the relationship between perioperative cognitive changes and MRI/fMRI imaging changes.
- Determine the relationship between perioperative CSF biomarker changes and perioperative MRI/fMRI imaging changes.
- Determine whether cognitive function differs in surgical patients vs non surgical controls over time.
- Determine whether CSF Alzheimer's disease marker levels differ over time in surgical patients vs non surgical controls over time.

4. INVESTIGATIONAL PLAN

4.1 Overall Plan

The objectives listed above are accomplished in eight parts:

1. **Site training/in-servicing.**
   The study personnel will be instructed to:
   1. Maintain a personnel signature log file
   2. Maintain a Correspondence log file (e-mail and phone)
   3. Maintain a file with:
      a. Study Protocol signed by Investigator
      b. Copy of IRB/ethics committee approval
      c. Copy of IRB/ethics committee approved consent form
      d. Copy of laboratory certification
      e. Copy of Investigator's Curriculum Vitae
      f. Copy of IRB/ethics committee membership that approved the study and name of the Chairman (if possible)
      g. Protocol and/or CRF amendments
      h. Copy of Training and Audit reports

4. Maintain an updated subject enrollment/completed log file
5. Maintain a file with the ID numbers of CRFs corresponding to serum/CSF samples
6. Maintain a file of completed CRFs and signed consent forms

2. Study initiation.

Study initiation will start with the enrollment of the first patient. Enrollment will commence only after the respective institution's Institutional Review Board or Ethics Committee has approved the protocol and copies of the documents listed in section 3.1.1.4a-h have been completed. CRFs will be completed and specimens will be collected.

3. Initial Lumbar Puncture and Blood Draw:

A lumbar puncture will be performed, within 1 hour prior to the scheduled start of anesthesia care, with a standard spinal anesthesia kit under strictly sterile conditions. In non-surgical controls, the first lumbar puncture will be performed within 1 month after the baseline cognitive testing session.

For both non-surgical controls and surgical patients in the study, the procedure itself will be identical. First, a procedural time out will be performed by the study anesthesiologist and the preoperative nurse or other non-study clinical personnel, or the clinical trial coordinator or clinical trials assistant, to verify the patient name, medical record number, date of birth, signed, dated, and timed study consent form, the procedure (lumbar puncture), and the site marking (at the interspace between the 4^{th} and 5^{th} lumbar spinous processes). The patient will then be placed in position with the back arched and the knees close towards the chest to open up the lumbar intervertebral spaces. The lumbar puncture site will first be sprayed with an over the counter numbing spray containing lidocaine, bupivacaine, or a similar local anesthetic.

As per standard of care, all personnel within the room will be wearing a hair net and protective mask. The L4-5 and L5-S1 interspaces will then be cleansed with sterile prep for 30 seconds. The study anesthesiologist will then perform the lumbar puncture using a standard spinal anesthesia kit per the manufacturer's directions under sterile conditions (sterile drape, sterile gloves and procedure) at either the L4-5 or L5-S1 interspace, depending on which interspace appears larger by palpation. If the lumbar puncture needle in the spinal anesthesia kit is found to bend excessively or otherwise function improperly, the study anesthesiologist may use an another spinal needle (also used under strictly sterile conditions), as per standard of care and good clinical practice for any neuraxial anesthetic procedure. The lumbar puncture needle will be accessed to obtain CSF only by an Anesthesiology Attending, fellow or senior resident, who will do so while wearing sterile gloves and in a sterile fashion. All CSF samples will be immediately placed into a sterile pre-chilled 15 ml polypropylene conical tube. The tube containing the CSF will be given to the study coordinator, who will immediately place it on ice.

Blood (10 ml) will be collected via sterile venipuncture, or from an arterial line or intravenous line placed prior to surgery.

4. **24 hr Post-Op, 6 week and 1 year Post-Op Lumbar Puncture and Blood Draws**
Lumbar puncture will be performed by an anesthesiology attending, fellow or senior resident at these time points in surgical patients, and at the cognate time intervals in non-surgical controls. Lumbar punctures will be performed as per our established protocol using a standard spinal anesthesia kit (see above). A pre-procedural time out will be conducted by the study anesthesiologist and the preoperative nurse or other study personnel as discussed above, verifying the patient's name, medical record number, date of birth, signed/dated/timed study consent form and procedure site (L4-5 interspace). At this point, the curtain to the preoperative space will be closed and/or the door to the room will be closed, and all personnel within this enclosed space will don a protective mask and hair net. If the patient is hospitalized overnight after the surgery, the 24 hr postop LP may be performed in the patient's hospital room, in the preoperative holding area, or other appropriate space for performing a sterile neuraxial procedure per good clinical practice guidelines. If the patient is discharged after surgery on the same day and does not spend the first night overnight in the hospital, then the 24 hr postoperative LP may be performed in the same location as the 6 week and 1 year postoperative LP's are performed or another safe location for performing sterile neuraxial anesthetic procedures per good clinical practice guidelines.

After this standard preoperative time out, the patient will be placed in position with the back arched and the knees close towards the chest to open up the lumbar intervertebral spaces. The preoperative nurse or study personnel will help position the patient, and will stand in front of the patient to ensure the patient does not fall forward off of the bed. Vital signs will be taken from standard American Society of Anesthesiology monitors (ECG, non invasive blood pressure cuff, and pulse oximeter) before and after the lumbar puncture.

The lumbar puncture site will first be sprayed with an over-the-counter numbing spray containing lidocaine, bupivacaine, or another similar local anesthetic. The site will then be cleaned with sterile iodine prep for 30 seconds. The study anesthesiologist will then perform the lumbar puncture at the L4-5 or L5-S1 interspace (depending on which space feels more open by palpation), using the spinal anesthesia kit per the manufacturers directions under sterile conditions (sterile drape, sterile gloves and procedure). 10 ml of CSF will be allowed to fall from the spinal needle into a sterile 15 ml pre-chilled polypropylene conical tube, or will be obtained from the needle with a sterile 10 ml syringe. The tube containing the CSF will be given to the study coordinator, who will immediately place it on ice. The spinal needle will then be removed from the patient's back and safely discarded in a sharps container, and a bandaid or tegaderm will be placed across the skin site. The anesthesiologist or study MD will then assist the patient in lying down on the bed, and the bed siderails will be raised to prevent the patient from falling out of the bed. The patient will then be continuously monitored for 15 minutes, or longer if deemed necessary by the study anesthesiologist.

Blood will drawn by sterile venipuncture or from an arterial or intravenous line if already in place.

5. Cognitive Testing.

   Neurocognitive Testing: Cognitive testing will occur preoperatively (baseline) at six weeks +/- 3 weeks after surgery, and at 1 year (+/- 2 months) after surgery in surgical patients, and at the cognate time intervals in the non-surgical controls. The following tests will be included in the assessment battery:

   a. Hopkins Verbal Learning Test - Revised (HVLT-R) - The Hopkins Verbal Learning Test is a word-list learning task used to assess memory. Over three trials, a 12-word list of three semantic categories is presented to the examinee who then recalls as many words as possible, in any order. After a 20-30 minute, a delayed recall and a delayed recognition trial are administered. For the delayed recall, the subject is asked to recall the word list from memory. The delayed recognition trial consists of a randomized list that includes 12 target words and 12 non-target words, 6 of which are drawn from the same semantic categories as the targets. Raw scores are derived for total recall (the total number of words recalled over the three learning trials), delayed recall (the number of words recalled after a delay), and delayed recognition trial (the number of true positive hits minus the number of false positive hits). Alternate forms will be used for the baseline, 6 week assessments and 1 year assessments to minimize the effect of practice.
   b. Randt Short Story Memory Test: The Randt requires subjects to repeat a brief paragraph that has been read aloud to them. Verbatim and gist recall is evaluated immediately and after a 30-minute delay. The test is used to assess discourse memory (immediate and delayed) and oral language comprehension.
   c. Modified Visual Reproduction Test from the Wechsler Memory Scale: This test measures short- and long-term figural memory and requires subjects to reproduce from memory several geometric shapes both immediately and after a 30-minute delay.
   d. Selected subtests from the WAIS-R:
      1. Digit Span: This is a test of short-term auditory memory and attention that requires subjects to repeat a series of digits that have been orally presented to them both forward and, in an independent test, in reverse order.
      2. Digit Symbol: This test measures psychomotor processing speed and attention and requires subjects to reproduce, within 90 seconds, as many coded symbols as possible in blank boxes beneath randomly generated digits, according to a coding scheme for pairing digits with symbols.
      3. WRAT 3 Reading Subtest: The WRAT-3 test assesses the basic reading skills. It has two alternative testing forms (blue and tan). One form is administered with the second form available if needed. Each reading test consists of 15 letters and 42 individual words printed on a plastic card. The examinee is asked to read the words loudly while the examiner follows a phonetic guide for each reading item. One point is given for each correct letter and word. A maximum of 57 points can be earned on either the blue or the tan form. The raw scores are recorded at the bottom of the word reading section.

4. Trail Making Test, Part A and B: Trails is a test of processing speed and attention. In Part A, subjects are required to connect a series of numeric circles as quickly as possible. In Part B, subjects connect a series of numeric and alphabetic circles in order (e.g., 1-A-2-B etc.) as quickly as possible.
5. Grooved Pegboard: Timed test of motor speed and coordination recommended by consensus panels on the assessment of cognitive decline after surgery.[26, 32] Used to control for generalized slowing in performance which might confound performance on some of the other timed neuropsychological tests.

e. Mini mental status exam: a brief 30-point questionnaire test that is used to screen for cognitive impairment, including simple questions and problems in a number of areas: the time and place of the test, repeating lists of words, arithmetic such as the serial sevens, language use and comprehension, and basic motor skills.
f. Subjects will also undergo the STOP-BANG brief questionnaire to assess sleep apnea risk during one of their study visits (preop, 6 weeks post op, or 1 year post-op).
g. Subjects will complete the visual analogue scale pain assessment at each study visit (preop, 6 weeks post op, and 1 year post op).
h. Subjects will also fill out a list of medications they are currently taking at each study visit.

The tests listed above will be recorded using a digital voice recorder for quality assurance purposes. Quality assurance activities will include reviewing of testing audio files, generating performance review documents, and providing feedback on accuracy and completeness. There is no risk to the study subject since patient identifiers will not be provided. Study subjects will not be identified by name on the recording. Only the study number, test date and initials of the tester will be provided on the digital file label. The link between study number and patient identifiers will be maintained. These files will be stored on locked computers within locked offices to which only authorized research personnel will have access. These files will be destroyed after the primary outcome analysis of this research study is complete.

Falls Assessment: All study patients (both surgical patients and non-surgical controls) will fill out a brief falls risk assessment questionnaire, which contains questions from the Elderly Falls Screening test (http://www.wcrtac-wi.org/uploads/Falls_Screening___Referral.pdf) and the Fall Risk Screening test (Tromp et al, J Clin Epidemiology, 2001), and questions about whether they have fallen recently. This questionnaire will be performed in person at the initial pre-operative study visit, and then will be mailed to patients for them to complete at home and bring to the 6 week and and 1 year post-op study visits. At each study visit, patients will complete the Timed-Up-And-Go test (TUG; a test of overall physical status). The TUG measures the time that it takes for a patient to get up from a seated position and walk ten feet, turn around and walk back to the chair and sit down. Additionally, the Romberg test (a test of

proprioception and balance) will be performed on each patient at each study visit. Any patient who feels unable to complete either the Timed Up and Go test or the Romberg test or who feels unsafe completing either test will be allowed to skip the test(s) in question, and this will be noted by study personnel.

Quality of Life Assessment: Our approach to measuring quality of life for this study involves the use of a battery of well-tested and well-validated instruments that together cover the relevant domains of interest. For this study, we propose to use an instrument that includes a measure of functional status derived for use in cardiovascular populations (the Duke Activity Status Index or DASI), psychological measures including depression (the CES-D), the State Trait Anxiety Inventory (STAI), the Hopkins Symptom Checklist (SCL-90), and social/role functioning (including employment status). Our current battery includes the Short Form 36 (SF 36) generic quality of life instrument developed by the Randt group from their longer instruments used in the Health Insurance Experiment and in the Medical Outcomes Study. Subjects will complete these questionaires during the baseline study visit, after completing all consent paperwork. These questionaires will then be sent to patients via the mail (electronic and/or regular mail) prior to the 6 week and 1 year study visits and study patients will be asked to complete the questionaires and bring them to these study visits, or the study subjectswill be given these questionaires at these study visits,

1. Neuroimaging Procedures: Up to 70 surgical subjects and 70 non-surgical controls will complete neuroimaging procedures at pre-operative baseline, and 6 weeks +/- 3 weeks after surgery, which will consist of high-resolution anatomic, perfusion and resting-brain fMRI sequences; all of which will be acquired on a 3-Tesla General Electric magnetic resonance scanner provided by the Brain Imaging and Analysis Center (BIAC) at Duke University. No contrast agent will be administered in these imaging procedures. Standard pre-scanning safety procedures will be conducted on the day of imaging appointments for final clearance to enter the BIAC magnet suite for testing. For those subjects with recent body implants appropriate for MR use and located below the neck, surgical records regarding the implant type will be obtained if available and/or relevant and filed with the BIAC staff. For subjects with bodily implants that are made out of non metallic materials (ie plastic or acrylic materials used for mesh placement in hernia repairs), records will not be required since these materials are non-metallic and thus MRI safe. Subjects who have bodily implants that are not MRI safe (per good clinical practice guidelines) will not undergo MRI scan procedures under any circumstances, but may still be included in the study and undergo cognitive testing and blood/CSF sampling. For subjects who have bodily implants that are made of MRI safe metallic materials (ie titanium), decisions about whether to scan the patient will be made based on good clinical practice guidelines based on the known properties of the implant materials as well as any formal MRI testing that has been performed of the implant in the MRI environment.
   Anatomical scanning will begin with a T1-weighted sagittal localizer series, after which high-resolution T1-weighted FSPGR, T2 FLAIR, T2/PD and diffusion-weighted (DTI) structural sequences will be acquired. These structural sequences are followed by a single, resting acquisition of an arterial spin labeling (ASL) sequence for the evaluation of resting cerebral perfusion. The ASL, T2 FLAIR and T2/PD sequences were included in an effort to account for potential influences of regional cerebral perfusion and ischemic burden on post-surgical functional brain change. These auxiliary imaging techniques will serve to provide potential covariates in subsequent analyses of longitudinal functional brain change and any association between standard neuropsychological assessment and resting-brain fMRI change variables. Before undergoing any of the imaging sequences, patients will be outfitted with MR-safe physiological recording devices to record pulse rate, respiration, galvanic skin response (GSR). All participants will be given an

opportunity to ask any questions prior to imaging, and additional opportunities for questions will be prompted between each of the individual imaging protocol sequences.

Neuroimaging Sequences

Anatomical imaging – A standard sagittal localizer series will be acquired at the beginning of the scanning procedure. To insure adequate resolution for tissue segmentation and manual ROI tracing, an axial, inversion-prepared 3D FSPGR sequence (TR 22, TE 5.4, TI 450, 20° flip, 256^2 matrix, 1 mm^3 voxels) will be used to acquire structural data with high-resolution. Additionally, to account for possible prior cerebrovascular compromise in the patient samples, an axial fluid-attenuated inversion recovery sequence (FLAIR; TR 11000, TE 149.5, TI 2250, 90° flip, 256^2 matrix, $1mm^2$ in-plane res., no-gap $2mm^3$ slices) and T2 proton density sequence (T2/PD; TR 3000ms, TE 25, 90° flip, 256^2 matrix, $1mm^2$ in-plane res., no-gap $2mm^3$ slices) will be collected to obtain volumetric estimates of white matter hyperintensities (if present). A 26-direction diffusion–weighted (DTI) sequence (TR/TE = 17000/83 ms, 90° flip, 256^2 matrix, $1mm^2$ in-plane res., no-gap $2mm^3$ slices) will be used to collect global maps of fractional anisotropy (FA), radial diffusivity (RD) and axial diffusivity (AD) for evaluation of microstructural post-operative white matter changes, often not visible on FLAIR or T2/PD sequences. Total time to complete anatomical scanning is estimated at 20 minutes.

Perfusion imaging – A modified FAIR sequence, in which the standard global inversion pulse is replaced by a spatially selective inversion pulse combined with QUIPSSII saturation pulses, will be used to acquire slices coplanar to the high resolution anatomical images. Tag and control images will be interleaved across 100 repetitions. Calibration scans, suppressing CSF and minimizing gray-white contrast, necessary for perfusion quantification, will also be performed. The basic parameters for the ASL series will be: TR 2500ms, TI1 600 ms, TI2 1600 ms, 90° flip angle, and minimum TE of 3 ms. Total time to complete perfusion imaging is estimated at 6 minutes.

Functional imaging – 34 contiguous slices parallel to the AC-PC sensitive to blood-oxygen level dependent (BOLD) contrast will be acquired coplanar to the high-resolution axial anatomical sequence using a SENSE spiral-in sequence developed by BIAC for the GE 3T magnet. The functional volumes will be acquired with a TR 3000ms, TE 30ms, FOV 24cm, 64^2 matrix, and a 60° flip angle. The resulting slice thickness will be 4mm, resulting in cubic 4 mm^3 isotropic voxels. Prior to temporal and spatial alignment, the first six volumes of each functional run will be discarded to correct for initial MR field variance. Two resting-brain functional runs using the above sequence parameters will be administered for a total estimated functional imaging scan time of 12 minutes.

In concert with the resting state fMRI scan sequence, a MRI-compatible physiological monitoring system (In-Vivo Research) provides continuous non-invasive measurement of pulse rate, respiration, and galvanic skin response (GSR). Physiological data collection will be used to correct for biological signal/noise in the fMRI neuroimaging data at post-processing, but it additionally provides the opportunity for greater safety and non-invasive patient monitoring during the study's neuroimaging procedures. Resting state fMRI data will be collected using a standard fMRI sequence similar to the other functional data acquired in this study.

6. EEG recording: Scalp recordings of electrical activity produced by the brain (electroencephalograms [EEG]) will be obtained during anesthesia and surgery, and for roughly 15 minutes before the start and after the end of general anesthesia.. Electrodes (metallic disks with wires attached) are used to record the electrical signals of the brain at the scalp. The electrodes are embedded in a commercially available elastic cap that is worn on the head. At the scalp electrode locations, the scalp is cleansed and prepared using a non-toxic electrically conductive gel that is placed between the electrode surface and the scalp. Standard EEG recording equipment is used that protects the volunteer from any hazard of electrical shock using isolated grounding procedures. Electrodes and caps are sterilized between uses by immersion in an anti-viral and anti-bacterial solution, as well as by washing in warm soap and water.

Application materials are discarded after every use, and new materials are used for each volunteer. EEG data will be analyzed to determine predictors of postoperative cognitive function and brain activity (as measured by fMRI scans), and to search for EEG predictors of postoperative cognitive dysfunction and or delirium.

7. Genotyping, CSF protein, and serum inflammatory protein analysis.

   Genotyping and inflammatory protein analysis will be conducted as described (James ML et al, Stroke, 2009 Feb;40(2):632-9; Wang H et al, Anaesth Intensive Care. 2009 Jan;37(1):38-45.). CSF protein analysis will be performed using Covance Elisa kits as described (Wilcock et al, J Neurosci. 2009 Jun 24;29(25):7957-65), or by the Alzheimers Disease Neuroimaging Initiative (ADNI) biomarker core facility at the University of Pennsylvania as previously described (Lo RY et al, Arch Neurol. 2011 Oct;68(10):1257-66).

8. Data analysis.
   Data will be analyzed using standard analysis programs including Microsoft Excel, GraphPad Prizm, and SPSS.

3.2 Study Population and Specimen Criteria

100 surgical patients that present for non-cardiac, non-neurologic surgical procedures at Duke and 100 non-surgical controls (community dwelling older adults) will be prospectively enrolled. Patients aged 60 and above who meet the criteria described below will be eligible for the study. Surgical patients will be 'screened' for potential enrollment upon evaluation in preoperative screening clinic, the preoperative optimization of surgical health (POSH clinic), and/or surgery clinic. Non-surgical controls will be identified from the Duke Aging Center Research Subject Registry and/or the Duke Bryan Alzheimer's Disease Research Center (ADRC) Research Subject Registry, both of which contain community dwelling individuals over the age of 50 who have already agreed to be contacted about studies to better understand aging-related disorders such as Alzheimer's disease. We will enroll subjects of both genders, and all ethnicities and racial groups. Subjects with bodily implants unsafe for MRI use or with a history of severe claustrophobia will not undergo MRI scans, although they may still participate in other parts of the study. Patients who receive systemic chemotherapy after the first cognitive testing session and before either the 6 week or 1 year post-operative cognitive testing session is performed will be excluded from the study, to avoid the confounding effects of systemic chemotherapy on cognitive testing. Similarly, in the unlikely event that a patient undergoes major head trauma during this time period, that patient will also be excluded from the study to avoid the confounding effects of major head trauma on post-operative cognitive testing. Only subjects who complete all cognitive testing sessions and CSF/blood sample draws will count towards the 100 surgical patients and 100 non-surgical controls enrollment target.

Inclusion Criteria:
- age 60 and above
- ability to speak English.

Exclusion Criteria:
- age less than 60
- inmate of a correctional facility (i.e. prisoners).
- Family or personal history of malignant hyperthermia.
- Patient unable to receive either propofol and/or isoflurane due to allergy or other specific contraindication, if in the surgical arm of the study. This exclusion will not apply to non-surgical controls. .
-Receiving systemic chemotherapy between the time of the two cognitive testing sessions.
-Major head trauma that occurs between the time of the two cognitive testing sessions.
-History of PE, DVT, or other diagnosed bleeding or clotting disorder.

Enrollment Process: Surgical patients will be initially approached in their hospital room (if they are admitted prior to surgery) and asked whether they are interested in hearing about the study. If the patient is not admitted prior to surgery, they will be approached in the pre-operative screening clinic, the preoperative optimization of senior health clinic (POSH), or one of the surgery clinics or approached via a phone call. Individuals on the Duke Aging Center research Subject Registry and/or the Duke Bryan ADRC Research Subject Registry will be approached via a phone call about participating as non-surgical controls. Non-surgical controls will also be recruited through the databases on Researchmatch.org and the Duke Clinical Research Unit Research Registry.

Patients will be consented by one of the study investigators or coordinators, all of whom are trained in subject enrollment. If the patient consents to participate in the study, the study coordinator will then set up an appointment for the patient to have preoperative or baseline cognitive testing.

If a subject is able to give informed consent to participate in the study but physically cannot sign the consent form (due to blindness, hand injury, or other physical impairment), the subject's legally authorized representative may sign the consent form on behalf of and at the direction of the subject. In such cases, the subject must be able to understand the potential risks, benefits and alternatives to study participation, and all questions and concerns of the subject will be addressed. Patients or their LAR will sign all consent paperwork prior to taking part in any study procedures or cognitive testing.

Informed consent will be obtained from each subject whenever possible. In those situations where a subject is not competent to give informed consent, then the legally authorized representative will provide the consent on behalf of the subject. We will initially use consent of the legally authorized representative for subjects deemed not

to have medical decision making capacity; however, should these subjects regain medical decision-making capacity after enrollment, they will be re-consented prior to continuation of participation in study.

The PI will withdraw LAR consented subjects if they display distress, are uncooperative, or if the LAR consented subject indicates in any way that he or she does not wish to proceed with the LP and other procedures. 2) the LAR should be present at study procedures to act as an advocate and to request withdrawal if/as needed. The subject's capacity to consent will be assessed at each research visit by a qualified licensed person per DUHS policy.

Power Analysis and Study Size:

100 surgical patients will be enrolled in this study, based on our power analysis showing that 100 patients provides >80% power to detect a Spearman correlation coefficient of 0.3 or greater (a low moderate correlation) between the perioperative change in CSF tau levels and the perioperative change in the continuous cognitive change index.

100 surgical patients (50 subjects per each treatment arm) will be enrolled, also because our power analysis also shows that 50 patients per group will need to be enrolled to obtain a >90% power of detecting a 50% increase in tau levels pre/post anesthesia with an alpha of 0.05 in patients treated with isoflurane versus those treated with propofol, given a variance (or sigma) of 75% in each group. This analysis is based on the changes and variance in tau levels previously described (Tang et al, 2011). This power analysis was performed using the online power calculator *http://www.cs.uiowa.edu/~rlenth/Power/* set on the two sample t test.

100 non-surgical controls will be enrolled in the study as well, based on a power analysis showing that 100 surgical patients and 100 non-surgical controls will provide >80% power to detect a 30% increase in tau levels in surgical patients vs non surgical controls at the 24 hr time point, with an alpha of 0.05, given a variance (or sigma) of 75% in each group. This analysis is based on the changes and variance in tau levels previously described (Tang et al, 2011).. This power analysis was performed using the online power calculator *http://www.cs.uiowa.edu/~rlenth/Power/* set on the two sample t test.

Randomization:

Subjects will be randomized to receive either inhalational anesthesia with isoflurane or intravenous anesthesia with propofol. Anesthesia providers will be instructed to run the propofol infusion through a clearly visible IV site, and to check the site not less than twice per hour to ensure that the IV has not infiltrated. In each group, anesthesia providers will be instructed to adjust the anesthetic agent to maintain a bispectral index (BIS) value between 40-60, unless otherwise dictated by the surgical procedure. Subjects, if any, deemed unsuitable for either inhaled or intravenous anesthesia and treated with the other anesthetic (i.e. intravenous anesthetic patients given isoflurane) will be excluded from the study analysis.

Specimens:
Whole blood specimens (10 ml total volume) will be collected prior to surgical incision (10 ml) and 24 hours later, as well as at the 6 week and 1 year follow up visits. Whole blood specimens will be collected with EDTA as the anticoagulant, obtained either from an arterial or intravenous catheter or from sterile venipuncture. CSF specimens will be collected when the lumbar puncture is performed prior to anesthetic induction (prior to primary surgical incision), and 24 hours later, as well as at the 6 week and 1 year follow up visits. The duration of anesthetic exposure shall be recorded for each subject.

3.3 Materials

A. Provided by the Investigator(s)

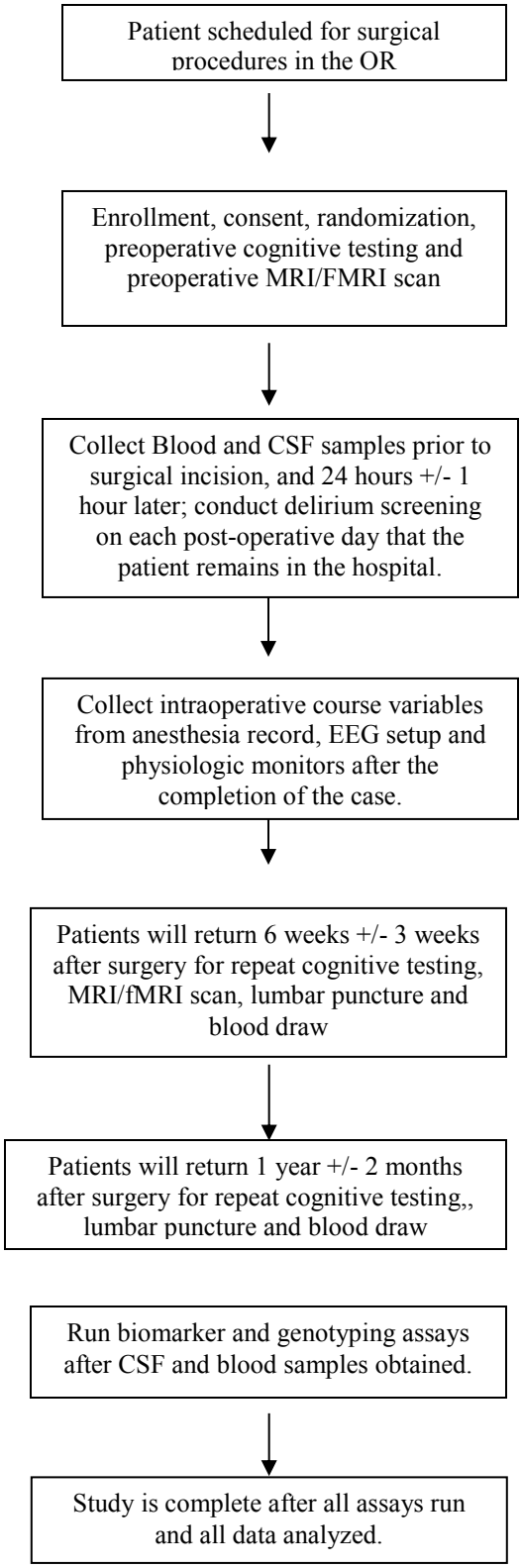
1. Study enrollment log.
2. Plasma specimens from each enrolled patient, obtained from whole blood using EDTA as the anticoagulant.
3. CSF specimens from each patient, obtained via lumbar puncture.
4. Completed Case Report Forms associated with the patient.
5. Raw data from laptop computer.
6. Cryovials for aliquoting plasma samples.
7. Freezer boxes for plasma samples.
8. Barcode Labels

3.4 Reports

CRFs and the plasma aliquots should be completed at every sample draw. The information required for completion of the information forms is provided on each form and must be completely filled out by the investigator or designee**.** Study personnel shall obtain the requisite patient consent forms to permit the use contemplated hereunder of all plasma aliquots and information collected from patients' clinical records or during the study, including the health and disease history information, for research and development purposes without compensation to the donor. Study personnel shall also prepare and maintain complete and accurate records of plasma aliquot sources, donor medical history, any infectious disease screening, together with any other records required by applicable laws and regulations**.**

4 PROCEDURE

4.4 Process Flow



4.5 Blood and CSF Sample Intervals

Blood and CSF will be collected at the following time points for subjects:

Blood (10 ml) and CSF (10 ml) will be collected within 1 hour of the scheduled anesthesia start time but prior to surgical incision, 24 hours +/- 1 hour later, 6 weeks +/- 3 weeks later, and 1 year +/- 2 months later.

4.6 CSF Sample processing, Storage and Transfer

1. Collect 10mL CSF, at each time point after enrollment, as described in section 4.2

2. Aliquot the CSF.

3. Using a single sheet of barcode labels, affix one barcode label to the collection tube and one barcode label to the appropriate area on the CRF. The labels on each sheet have the same 5 digit number and are followed by a unique letter. Each collection tube should be associated with a different 5-digit number.
4. Label the CSF tube appropriately and store in provided box in a -80°C or below freezer.

4.7 Blood Sample Processing, Storage, and Transfer

Extra precautions are necessary to ensure the stability of the analyte in plasma and whole blood specimens. The following protocol should be followed:

1. Collect a 10 mL EDTA coated whole blood Vacutainer tube (purple top) from each patient prior to anesthesia induction, and another 10 ml EDTA coated whole blood vacutainer tube (purple top) 24 hours later. Any combination of tube sizes may be used to obtain the specified volume of blood collected.

2. Gently invert the collection tube eight times to ensure dissolution of the anticoagulant, avoiding prolonged contact with the rubber stopper.

3. Using a single sheet of barcode labels, affix one barcode label to the collection tube and one barcode label to the appropriate area on the CRF. The labels on each sheet have the same 5 digit number and are followed by a unique letter. Each collection tube should be associated with a different 5-digit number.

4. Place the sample on ice, then centrifuge at 2000 RPM for 15 minutes. The upper plasma phase will be pipetted off and stored in 1 ml aliquots at -80 degrees Celcius. The lower blood phase and white cell phase shall be stored at -80 degrees Celcius as well.

4.8 Data Collection

CRFs will be completed at the time of enrollment and during the operative procedure. Data collected will incorporate variables reported in the subject's medical record and anesthetic record as dictated by standard of care practices and will include, but not be limited to, vital signs, brain oxygenation, imaging studies, neurological exam, cerebral blood flow measurements, medications, presence of mechanical ventilation and/or tracheostomy, culture data, laboratory profiles, ECGs, and type of diet.

4.9 Data & Safety Monitoring

Both arms of this study involve standard of care anesthetic regimens. The long term consequences of acute perioperative changes in Alzheimer's disease markers are unclear. Thus, we have no plans to stop the study early if we find larger changes (or increases) in these markers in one study arm or the other.

All subjects will be given the contact information for Dr Miles Berger or the study physician on call, and encouraged to be in touch at any time if they develop a headache after the lumbar puncture or any other concerning signs or symptoms. If the study subject calls and reports that he or she has developed a headache related to the lumbar puncture (ie a post-dural puncture headache), the study physician will assess whether this headache is likely related to the lumbar puncture. This will be done by asking the patient questions such as: "Does the pain gets worse with standing? Does it improve with laying flat? Do bright lights or loud sounds make the headache worse?"

If Dr Berger or the study physician on call diagnoses the patient with a post-dural puncture headache (i.e. a headache related to the lumbar puncture), then the patient will initially be treated conservatively- i.e. the patient will be encouraged to lie flat, to increase his or her caffeine consumption, and to increase overall fluid consumption. If these measures are not successful in improving the headache, then Dr Berger or the study physician on call may perform an epidural blood patch free of charge to improve the patient's headache,

The accuracy of data analysis will be verified by each investigator going over the primary data and analysis thereof. The accuracy and precision of laboratory assays will be assured with the measurement of appropriate controls for each assay. Investigators conducting the laboratory assays will be blinded as to a subject's (and sample's) study arm assignment, thus eliminating the possibility of investigator bias while conducting these assays.

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