New Technologies to determine Carotid Plaque Vulnerability: A Pilot Study to Assess Contrast-Enhanced Ultrasound (CEUS) and Shear Wave Elastography (SWE) in Patients with Significant Carotid Plaques

NCT03335033

8/24/2018

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IRB Minimal Risk Protocol Template

Note: If this study establishes a human specimen repository (biobank) for research purposes, do not use this template. Use the Mayo Clinic Human Specimen Repository Protocol Template found on the IRB home page under Forms and Procedures at [redacted]

First-time Use: Use this template to describe your study for a new IRB submission.

1. Complete the questions that apply to your study.
2. Save an electronic copy of this protocol for future revisions.
3. When completing your IRBe application, you will be asked to upload this document to the protocol section.

Modification: To modify this document after your study has been approved:

1. Open your study in IRBe. Click on the study 'Documents' tab and select the most recent version of the protocol. Save it to your files.
2. Open the saved document and activate "Track Changes".
3. Revise the protocol template to reflect the modification points , save the template to your files
4. Create an IRBe Modification for the study and upload the revised protocol template.

General Study Information

Principal Investigator: Matthew W. Urban

Study Title: New Technologies to determine Carotid Plaque Vulnerability: A Pilot Study to Assess Contrast-Enhanced Ultrasound (CEUS) and Shear Wave Elastography (SWE) in Patients with Significant Carotid Plaques

Protocol version number and date: 17-001863 8/24/2018

Research Question and Aims

Hypothesis: Identification of vulnerable atherosclerotic plaques can be evaluated based on intraplaque neovascularization as detected with contrast-enhanced ultrasound (CEUS) and/or by stiffness as measured by ultrasound-based shear wave elastography (SWE).

Aims, purpose, or objectives:
Aim 1: Evaluate the use of CEUS and SWE in patients undergoing carotid endartectomy
Aim 2: Evaluate the relationship of CEUS and SWE with subsequent major adverse cardiovascular events in patients with intermediate sized plaques.

The goal of this work is to determine if characterization of vulnerability of carotid plaques can be improved by measuring if there is neovascularization in the plaque with the CEUS method or by measuring the mechanical stiffness of the plaque using the SWE method. We will test these in plaques of significant size (stenosis of > 50%)

Background (*Include relevant experience, gaps in current knowledge, preliminary data, etc.*):

Stroke is a debilitating and fatal condition that is often caused by embolic blockage of blood vessels in the brain. About 795,000 people have new or recurrent stroke each year in the US and 87% of those are ischemic, caused by athero-emboli [1]. The source of the embolism can often be constituent materials from atherosclerotic plaques that form in the carotid artery. Characterization of the vulnerability of the plaque to rupture is therefore very important to reduce the risk of stroke. Noninvasive imaging modalities such as ultrasound, magnetic resonance imaging (MRI) and computed tomography (CT) have been used to determine plaque morphology and constituents [2-8]. MRI has high sensitivity for determining plaque constituents and results compare strongly with histology, but MRI is expensive and may not be widely available [3, 4, 9-12]. Ultrasound has many attractive attributes in that it is relatively inexpensive, fast, widely available, can be used for serial assessment, and does not use ionizing radiation. Echogenicity and simple geometrical measurements with ultrasound are insufficient for assessing plaque vulnerability [3, 4]. Because ultrasound is widely used for plaque detection, new noninvasive ultrasound imaging tools for plaque mechanics and neovascularization evaluation could be an integral part of determining plaque vulnerability which would affect cardiovascular disease risk stratification and therapy.

Plaque rupture and healing are dynamic in nature. Histological studies have recognized that a number of molecular changes including inflammation, angiogenesis, lipid accumulation and thrombosis occur at sites of atherosclerotic plaque development, and result in further physiologic progression, and ultimately, rupture [13, 14]. Vulnerable plaques in the carotid and coronary arteries are characterized by a thin fibrous cap, intraplaque hemorrhage, inflammatory activity, and a large lipid-rich necrotic core [15-17]. Currently, clinical decisions are made on the basis of ultrasound measurements of stenosis severity and Doppler-based flow velocities [18]. When the stenosis exceeds 70% diameter, then stenting or endarterectomy interventions are often used. However, patients with plaques that produce stenosis of 50-99% are challenging in terms of clinical decision making and management is controversial, especially in the absence of symptoms. In addition to the causal nature of stroke related to carotid plaques, patients with vulnerable plaques in the carotid are also typically prone to other forms of cardiovascular disease, in particular major adverse cardiovascular events (MACEs).

Importantly, stiffness of the plaque has been correlated to plaque vulnerability [19-35]. Plaque stiffness can be measured using shear wave elastography (SWE) methods. Methods for measuring tissue stiffness in large soft tissues and carotid plaques have been developed in the Mayo Clinic Ultrasound Research Laboratory [36-38]. Intraplaque neovascularization (IPN) can be evaluated with carotid contrast-enhanced ultrasound (CEUS). Multiple studies involving CEUS have shown that detection of microbubbles within the plaque can detect IPN, and the increase of signal intensity in the plaque can be visually scored [39-41]. A meta-analysis and systemic review evaluated the diagnostic accuracy of detecting IPN and the prognostic value of prediction of cerebrovascular and cardiovascular events by CEUS [42]. The contrast agent is confined to the vasculature, and to our knowledge, there are no reports that the contrast agent has caused plaque disruption and subsequent stroke. Combining SWE and CEUS imaging methods along with regular B-mode characteristics may provide improved techniques to evaluate plaque vulnerability.

Study Design and Methods

Methods: *Describe, in detail, the research activities that will be conducted under this protocol*:

Patient selection: We will prospectively recruit 80 patients for this study from patients being seen in the Gonda Vascular Center. Patients with known plaques will be screened before a scheduled appointment. Alternatively, patients undergoing an ultrasound examination in the Gonda Vascular Center may also be identified for recruitment. Patients who are will be undergoing a carotid endarterectomy will be approached for recruitment. Additionally, patients are going to be scanned and have a known moderate (50-69%) stenosis or a newly discovered moderate stenosis will be approached for recruitment. We will prospectively enroll 20 patients that are undergoing carotid endarterectomy and 60 cardiovascular high-risk patients with moderate or severe (50%-99% diameter) stenosis carotid plaques from the Gonda Vascular Center practice for evaluation with SWE and CEUS. The main indications of high-risk patients are multiple cardiovascular risk factors, carotid bruit, known atherosclerotic disease in coronary (>50% stenosis confirmed by angiography), or peripheral artery disease (according to medical history).

Ultrasound protocol: A standard carotid US examination using Duplex imaging will performed using a 9 MHz linear array transducer to obtain longitudinal and transverse images. All ultrasound measurements will be made by a trained registered vascular technologist. Severity of stenosis of the plaque will be measured based on established protocol and guidelines outlined in [44]. SWE will be performed with the GE Logiq E9 scanner acquiring images of each plaque in the longitudinal and transverse imaging planes. In each plane 10 measurements will be made and the in-phase/quadrature (IQ) data will be saved for further analysis. The plaque area in the imaging plane will be user-defined to extract the wave velocity values for a ROI analysis. A mean and median of repeated measurements will be calculated. Additionally, the calculated wave motion will be analyzed further for characterization of the plaque.

CEUS will be performed using low mechanical index (0.17-0.20) imaging during ultrasound contrast agent intravenous infusion. The ultrasound contrast agent that will be used is Lumason (Bracco Diagnostics, Inc). Real-time cine-loop including longitudinal and transverse images obtained at least 3 seconds before and 5 minutes after the appearance of the contrast effect in the lumen of the carotid artery will be acquired and digitally stored for offline analysis. IPN will be visually graded according to microbubble location and kinetics: G0: no visible microbubbles within the plaque; G1: moderate microbubbles confined to the shoulder and/or adventitial side of the plaque; or G2: extensive microbubbles throughout the plaque, with clear visible appearance of bubble moving into the plaque core. We will also use a 3D ultrasound transducer to evaluate the feasibility of using US images with contrast to assess the residual diameter of the diseased vessel. Specifically, two 3D acquisitions of each lesion would be obtained after contrast has been injected. These will be acquired with the RSP6-16-D mechanical 3D probe. One acquisition would be done with the vessel longitudinally oriented in the acquired image plane, and the other would orient the vessel trans-axially.

Endarterectomy Sample Analysis: If the surgeon sends the endarterectomy sample for pathological examination, we will analyze slides in the Tissue Registry. If the endarterectomy sample is not sent for pathological examination, fresh tissue from endarterectomy would be collected during the patient's surgery. If possible, the whole atherosclerotic plaque would be collected, but half the available specimen would also be suitable. The sample will be fixed in 10% neutral buffered formalin and subsequently embedded in paraffin (FFPE). Slides will be processed identically, whether processed clinically or for the purposes of research. Several slides (up to 6) with various stains will be processed for each block. These stains include hematoxylin and eosin (H & E), Verhoeff-van Gieson (VVG), Masson's trichrome, and CD31 antibody. The slides will be 5 microns thick for each stain. Slides will be stored in Dr. Urban's laboratory. Dr. [redacted] (a cardiovascular pathologist) will review the slides for this study.

Follow-up: We will perform follow-up calls with subjects at 6, 12, 24, and 36 months to ask about occurrence of major adverse cardiovascular events (MACEs).

Resources: *Describe the available resources to conduct the research (personnel, time, facilities, mentor commitment, etc.)*:

We will conduct these studies in the Gonda Vascular center with General Electric Loqiq E9 ultrasound scanners to perform ultrasound imaging, CEUS measurements and SWE measurements. Our team is comprised of investigators from the Department of Cardiovascular Diseases and the Department of Radiology.

As this is an investigational use of Lumason in the carotid artery for detection of IPN in the plaques, the results of these studies will not be included in subjects' medical records nor disclosed to their care providers in order to prevent these results influencing the subjects' clinical care.

☐ (1a) This is a multisite study involving Mayo Clinic and non Mayo Clinic sites. *When checked, describe in detail the research procedures or activities that will be conducted by Mayo Clinic study staff.*

☐ (1b) Mayo Clinic study staff will be engaged in research activity at a non Mayo Clinic site. *When checked, provide a detailed description of the activity that will be conducted by Mayo Clinic study staff.*

Subject Information

Target accrual is the proposed total number of subjects to be included in this study at Mayo Clinic. A "Subject" may include medical records, images, or specimens generated at Mayo Clinic and/or received from external sources.

Target accrual: 80

Subject population (children, adults, groups): Adults with atherosclerotic plaques in their carotid artery or arteries

Inclusion Criteria: Adult male and non-pregnant women 18 years old or older, Subject has carotid plaque with > 50% stenosis, Subject is able to understand the study procedure and provide informed consent

Exclusion Criteria: Subject is pregnant or breast feeding; Subject is unable to provide informed consent. Subject has contraindication to the use of the Lumason contrast agent.

Research Activity

Check all that apply and complete the appropriate sections as instructed.

1. ☒ **Drug & Device**: Drugs for which an investigational new drug application is not required. Device for which (i) an investigational device exemption application is not required; or the medical device is cleared/approved for marketing and being used in accordance with its cleared/approved labeling. (Specify in the Methods section)

2. ☐ **Blood**: Collection of blood samples by finger stick, heel stick, ear stick, or venipuncture.

3. ☒ **Biological specimens other than blood**: Prospective collection of human biological specimens by noninvasive means that may include: urine, sweat, saliva, buccal scraping, oral/anal/vaginal swab, sputum, hair and nail clippings, etc.

4. ☒ **Tests & Procedures:** Collection of data through noninvasive tests and procedures routinely employed in clinical practice that may include: MRI, surface EEG, echo, ultrasound, moderate exercise, muscular strength & flexibility testing, biometrics, cognition testing, eye exam, etc. (Specify in the Methods section)

5. ☒ **Data** (medical record, images, or specimens): Research involving use of existing and/or prospectively collected data.

6. ☐ **Digital Record**: Collection of electronic data from voice, video, digital, or image recording. (Specify in the Methods section)

7. ☐ **Survey, Interview, Focus Group:** Research on individual or group characteristics or behavior, survey, interview, oral history, focus group, program evaluation, etc. (Specify in the Methods section)

☐ NIH has issued a *Certificate of Confidentiality* (COC). *When checked, provide the institution and investigator named on the COC and explain why one was requested.* _____

Biospecimens – Categories 2 and 3

(2) Collection of blood samples. When multiple groups are involved copy and paste the appropriate section below for example repeat section b when drawing blood from children and adults with cancer.

a. **From healthy, non-pregnant, adult subjects who weigh at least 110 pounds**. For a minimal risk application, the amount of blood drawn from these subjects may not exceed 550ml in an 8 week period and collection may not occur more frequently than 2 times per week.
   Volume per blood draw: _____ml
   Frequency of blood draw (e.g. single draw, time(s) per week, per year, etc.) _____

b. **From other adults and children considering age, weight, and health of subject.** For a minimal risk application, the amount of blood drawn from these subjects may not exceed the lesser of 50 ml or 3 ml per kg in an 8 week period, and collection may not occur more frequently than 2 times per week.
   Volume per blood draw: _____ml

Frequency of blood draw (e.g. single draw, time(s) per week, per year, etc.) _____

(3) Prospective collection of biological specimens other than blood: _____

Review of medical records, images, specimens – Category 5

For review of existing data: provide a date range or an end date for when the data was generated. The end date can be the date this application was submitted to the IRB. Example: *01/01/1999 to 12/31/2015* or all records through *mm/dd/yyyy*.

Date Range:

Check all that apply (data includes medical records, images, specimens).

☐ (5a) Only data that exists before the IRB submission date will be collected.

☒ (5b) The study involves data that exist at the time of IRB submission **and** data that will be generated after IRB submission. Include this activity in the Methods section.
Examples

- The study plans to conduct a retrospective chart review and ask subjects to complete a questionnaire.
- The study plans to include subjects previously diagnosed with a specific disease and add newly diagnosed subjects in the future.

☐ (5c) The study will use data that have been collected under another IRB protocol. Include in the Methods section and enter the IRB number from which the research material will be obtained. *When appropriate, note when subjects have provided consent for future use of their data and/or specimens as described in this protocol.*

Enter one IRB number per line, add more lines as needed

☐ Data ☐ Specimens ☐ Data & Specimens _____

☐ Data ☐ Specimens ☐ Data & Specimens _____

☐ Data ☐ Specimens ☐ Data & Specimens _____

☐ (5d) This study will obtain data generated from other sources. Examples may include receiving data from participating sites or an external collaborator, accessing an external database or registry, etc. Explain the source and how the data will be used in the Methods section.

☐ (6) Video audio recording: *Describe the plan to maintain subject privacy and data confidentiality, transcription, store or destroy, etc.*


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HIPAA Identifiers and Protected Health Information (PHI)

Protected health information is medical data that can be linked to the subject directly or through a combination of indirect identifiers.

Recording identifiers (including a code) during the conduct of the study allows you to return to the medical record or data source to delete duplicate subjects, check a missing or questionable entry, add new data points, etc. De-identified data is medical information that has been stripped of all HIPAA identifiers so that it cannot be linked back to the subject. De-identified data is **rarely** used in the conduct of a research study involving a chart review.

Review the list of subject identifiers below and, if applicable, check the box next to each HIPAA identifier being recorded at the time of data collection or abstraction. Identifiers apply to any subject enrolled in the study including Mayo Clinic staff, patients and their relatives and household members.

Internal refers to the subject's identifier that will be recorded at Mayo Clinic by the study staff.
External refers to the subject's identifier that will be shared outside of Mayo Clinic.

Check all that apply:	INTERNAL	EXTERNAL
Name	X	
Mayo Clinic medical record or patient registration number, lab accession, specimen or radiologic image number	X	
Subject ID, subject code or any other person-specific unique identifying number, characteristic or code that can link the subject to their medical data	X	
Dates: All elements of dates [month, day, and year] directly related to an individual, their birth date, date of death, date of diagnosis, etc. **Note:** Recording a year only is not a unique identifier.	X	
Social Security number		
Medical device identifiers and serial numbers		
Biometric identifiers, including finger and voice prints, full face photographic images and any comparable images		
Web Universal Resource Locators (URLs), Internet Protocol (IP) address numbers, email address		
Street address, city, county, precinct, zip code, and their equivalent geocodes		
Phone or fax numbers		
Account, member, certificate or professional license numbers, health beneficiary numbers		
Vehicle identifiers and serial numbers, including license plate numbers		
Check 'None' when none of the identifiers listed above will be recorded, maintained, or shared during the conduct of this study. (exempt category 4)	☐ None	☒ None

Data Analysis

Power analyses and study endpoints are not required for minimal risk research, pilot or feasibility studies.

☐ No statistical information. *If checked, please explain*:

Power Statement: We will study 20 participants that are scheduled to undergo carotid endarterectomy. This is regarded as a pilot study. In the second aim we are studying 60 subjects with CEUS and SWE and performing follow-up to determine if any major adverse cardiovascular events (MACEs) occur at 6 and 12 months after the tests are performed. Follow-up will be continued for an additional three years after the completion of scanning. Given the 26% MACE rate in patients with known coronary artery disease who have echolucent carotid plaques and the 40% MACEs rate in cardiovascular high-risk patients with progressive carotid plaques during 3-year follow-up [43, 44], we will recruit 60 patients for this Aim.

Data Analysis Plan: Baseline and demographic descriptive analysis including age, gender, parity, weight, height, Body mass index (BMI), body surface area (BSA), cardiovascular risk factors, will be presented. Counts and percentages will be presented for categorical variables, while means ± standard deviations and 95% confidence intervals will describe continuous variables for which normality assumptions are met. If the distribution of a continuous parameter is skewed, transformations will be considered, or medians and interquartile ranges (IQR) will be presented. For all analyses we will use a two-sided P value less than 0.05 to be significant. All statistical analyses will be performed using JMP version 12 (SAS Inc., Cary, NC).

Safety on the use of ultrasound contrast agent will be also evaluated and reported as (%) of adverse events (categorized as minor or serious adverse events). The IPN will be counted and displayed as category data. Continuous variables will be compared between 2 groups using unpaired t-tests. For comparison of continuous variables in ≥3 groups, one-way analysis of variance (ANOVA) followed by a Scheffé test will be performed. Frequencies will be compared using chi-square analysis. The correlation between 2 continuous variables will be determined using a Pearson linear regression analysis. The diagnostic accuracy of CEUS and SWE when compared with histologic results will be determined by the area under the receiver operating characteristic (ROC) curve. Diagnostic accuracy of plaque vulnerability for each threshold will be summarized by a series of 4 x 4 tables to calculate the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). Youden's index will be used to select an optimal threshold for the diagnosis of vulnerable plaque.

The relationship between cardiovascular risk factors and the grade of plaque neovascularization will be examined using multiple logistic regression analysis. The model discrimination (c-statistic) will be compared to models including only the CEUS and SWE parameters and the combination of traditional risk factors and CEUS and SWE parameters.

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