
# vascuCAP™

# *Q-CAMP: Quantitative CArdiovascular Magnetic Resonance Imaging and Profiling of Atherosclerotic Lesions*

## Seeing Beyond the Image in Vascular Disease

**Program Reference Number: 0305**

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**LSUHSC-NO Institutional Review Board**

**Federal Wide Assurance 00002762 Registration # 00000177**

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## COPYRIGHT



## STATEMENT OF COMPLIANCE

The study will be conducted in accordance with the International Conference on Harmonisation guidelines for Good Clinical Practice (ICH E6), the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46), and the NHLBI Clinical Terms of Award. All personnel involved in the conduct of this study have completed human subjects protection training.

# REVISION RECORD

| Rev No. | Date | Author | Description |
|---|---|---|---|
| 1.0 | 20 Dec 2013 | Andrew J. Buckler | First version submitted for FDA review and starting point for filling in operational details. |
| 2.0 | 12 Mar 2014 | Andrew J. Buckler | Formal approval copy for IRB which incorporates FDA feedback and fleshes out operation details for start of study. |

## SIGNATURE PAGE

The signature(s) below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this study will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

LSHSC Principal Investigator:

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| ADC | Apparent Diffusion Coefficient |
| AE | Adverse Event/Adverse Experience |
| CFR | Code of Federal Regulations |
| CRF | Case Report Form |
| CSOC | Clinical Study Oversight Committee |
| DCC | Data Coordinating Center |
| DCE | Dynamic Contrast Enhanced |
| DHHS | Department of Health and Human Services |
| DSMB | Data and Safety Monitoring Board |
| DWI | Diffusion Weighted Imaging |
| ESS | Endothelial Shear Stress |
| Fast SE | Fast Spin Echo |
| FFR | Federal Financial Report |
| FWA | Federalwide Assurance |
| GCP | Good Clinical Practice |
| HIPAA | Health Insurance Portability and Accountability Act |
| ICF | Informed Consent Form |
| ICH | International Conference on Harmonisation |
| IRB | Institutional Review Board |
| ISM | Independent Safety Monitor |
| MOP | Manual of Procedures |
| MRI | Magnetic Resonance Imaging |
| N | Number (typically refers to participants) |
| NHLBI | National Heart, Lung, and Blood Institute, NIH, DHHS |
| NIH | National Institutes of Health |
| NLDR | Non-Linear Dimensionality Reduction |
| OHRP | Office for Human Research Protections |
| OHSR | Office of Human Subjects Research |
| PI | Principal Investigator |
| QA | Quality Assurance |
| QC | Quality Control |
| SAE | Serious Adverse Event/Serious Adverse Experience |

| | |
|---|---|
| SMC | Safety Monitoring Committee |
| SOP | Standard Operating Procedure |
| SS-EPI | Single Shot Echo Planar Imaging |
| TOF | Time of Flight |
| TSE | Turbo Spin Echo |
| US | United States |

# PROTOCOL SUMMARY

**Title:** Q-CAMP: **Q**UANTITATIVE **CA**RDIOVASCULAR **M**AGNETIC RESONANCE IMAGING AND **P**ROFILING OF ATHEROSCLEROTIC LESIONS

**Précis:** We use MRI to evaluate the extent, as well as, the structure, composition, and functional aspects of atherosclerotic plaques in human carotid and femoral arteries in patients scheduled to undergo an endarterectomy of the aforementioned vascular beds as part of their routine clinical care. The endarterectomy specimens removed at surgery will allow a direct comparison between the MRI information obtained prior to the surgery and the histopathological analyses of the arterial specimens. The vascuCAP™ measurements of structural and functional features of the arterial wall will be performed in patients undergoing endarterectomy. Measured results will be compared in a blinded fashion with histology to assess performance of plaque profiling and build a pilot prediction model for risk scoring.

**Objectives:**

Primary:

I. Measure the bias, precision, and reproducibility of each of the functional analytes as measured by vascuCAP™ with respect to histopathologic results as ground truth, and assess their linear region and limits of quantitation.

II. Establish performance characteristics of the structural and hemodynamic analytes, where an objective truth standard is lacking.

Secondary:

III. Classify the target lesion according to whether the analytes suggest it is not a plaque, or if it is, which of the 8 AHA types best fits the analyte profile. Assess the accuracy of vascuCAP™'s classification against the histopathology classification.

**Population:** >=110 patients (80 carotid patients, and 30 femoral patients) scheduled for endarterectomy.

**Sites:**

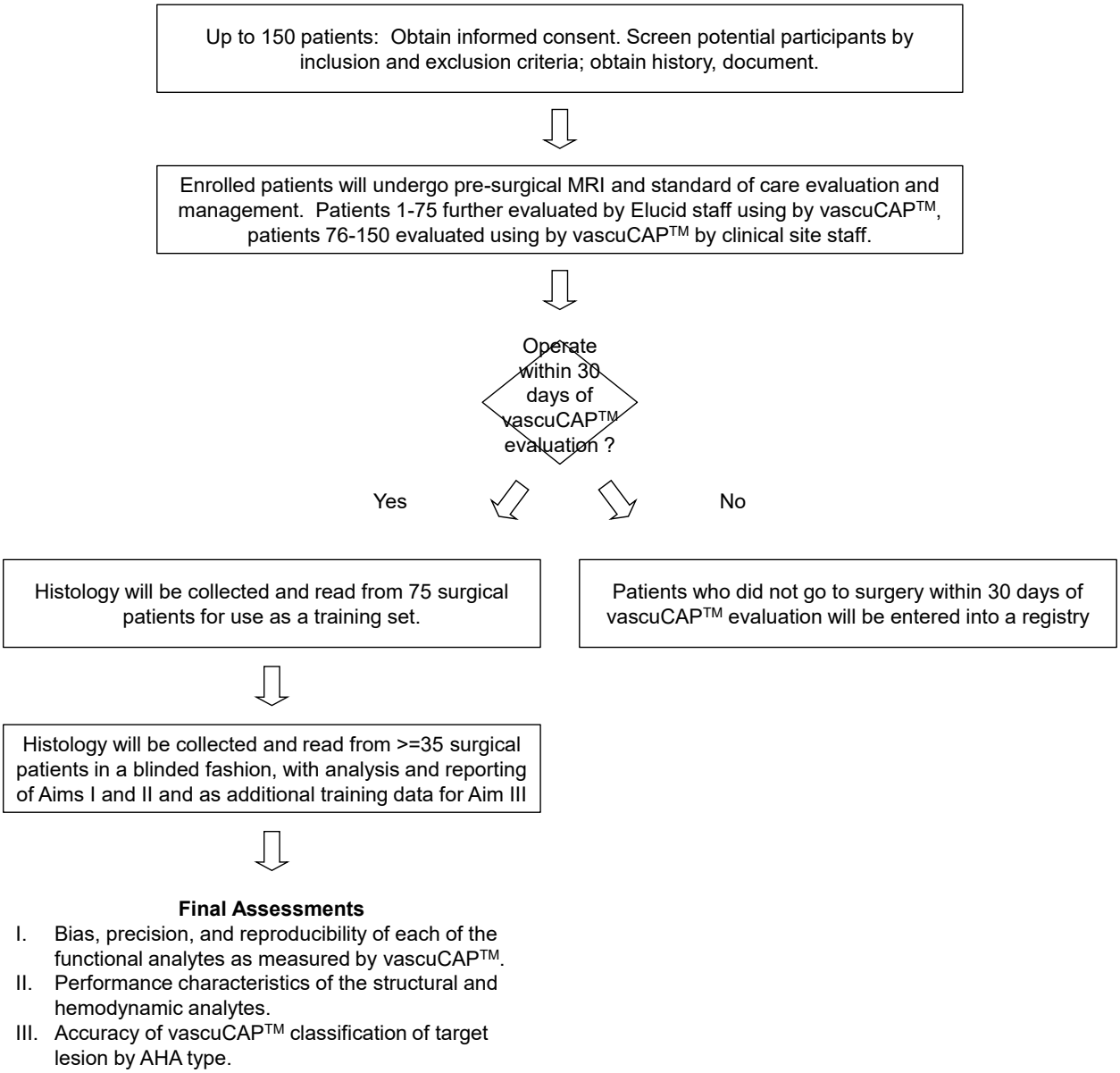
1. West Jefferson Medical Center, 1101 Medical Center Blvd, Marrero, LA 70072, (504) 347-5511
2. LSU ILH, 2021 Perdido St, New Orleans, LA 70112, (504) 903-3000

This study will be conducted within the LSU Department of Vascular Surgery at the above stated locations. Patients will be seen throughout the length of the study in both hospital and clinic settings.

**Study Duration:** 18 months for primary and secondary objectives.

**Estimated Time to Complete Enrollment:** 12 months

Schematic of Study Design:



# 1 KEY ROLES AND CONTACT INFORMATION

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**Institutional Review Board:** LSUHSC-NO Institutional Review Board, Federal Wide Assurance 00002762 Registration # 00000177

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# 2 INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

MRI is a safe, painless, and non-invasive way to image the body interior without harmful radiation. MRI provides superior information to conventional imaging studies because it can provide additional information regarding the chemical makeup of the atherosclerotic plaque. We have recently conducted imaging studies in a specialized rabbit model of atherosclerosis [1-5] that have served as a scientific rationale for the development of a computerized image analysis toolkit, vascuCAP™ [6].

## 2.1 Background Information

The purpose of this study is to determine the magnetic resonance imaging characteristics of clinically symptomatic, atherosclerotic plaques and compare them to histopathological analyses of arterial endarterectomy specimens obtained after surgery. Atherosclerosis is a response to chronic vascular injury that results in a dysregulated, vascular inflammation fundamentally associated with abnormalities of lipid metabolism. Current commonly employed modalities utilized to image atherosclerosis, such as invasive catheterization, fail to identify plaque components and poorly predict the likelihood of the plaque to produce acute ischemic events (instability). MRI provides unparalleled soft-tissue resolution non-invasively and has the potential to characterize plaque structural and functional components without invasive procedures.

**Table 1: Analytes interrogated by vascuCAP™, understood as a multiplex assay**

| Analyte | Description | Type and Units | Truth Standard |
|---|---|---|---|
| **Lipid Core** | Distinguished by presence of lipids, intermixed with extracellular matrix fibers, and/or necrotic tissue. | Expressed by burden in $mm^3$ and categorical variables for degree and uniformity using a 0/1/2 scale. | Histopathology considered as ground truth. |
| **Fibrosis** | Intimal presence of dense, homogeneous/organized collagen extracellular matrix with smooth muscle cells/fibroblasts embedded therein, but no appreciable lipid or necrotic tissue. | Expressed by volume in $mm^3$ and categorical variables for degree and uniformity using a 0/1/2 scale. | Histopathology considered as ground truth. |
| **Intra-plaque hemorrhage** | Presence of erythrocytes in the deeper regions of the plaque, with or without communication to the lumen or neovasculature. | Expressed by burden in $mm^3$ and confidence in %. | Histopathology considered as ground truth. |
| **Inflammation** | Accumulations of macrophages and lymphocytes in the deeper regions of the plaque that may bridge the neointima, and the media of the vessel | Expressed by burden in $mm^3$ and categorical variables for degree and uniformity using a 0/1/2 scale. | Histopathology considered as ground truth. |
| **Vascular Leak** | Composed of endothelial permeability, neovascularization, necrosis, and collagen breakdown.) | Expressed by distribution volume in $mm^3$ and categorical variables for degree and uniformity using a 0/1/2 scale. | Histopathology considered as ground truth. |
| **Calcification** | Intimal/medial presence of calcification with osteoblasts present therein, but | Expressed by burden in $mm^3$ and categorical variables for | Histopathology considered as |

| Analyte | Description | Type and Units | Truth Standard |
|---|---|---|---|
| | containing no appreciable lipid or necrotic tissue. | degree and uniformity using a 0/1/2 scale. | ground truth. |
| **Remodeling Ratio** | Calculated as the ratio of vessel area with plaque to reference vessel wall area without plaque | Expressed with value less than 1 for inward remodeling and greater than 1 for outward remodeling | Calculations from manual contour by radiologist as an imperfect(subjective) reference standard |
| **% Stenosis** | Calculated as the 1 - (ratio of lumen area with plaque to reference lumen area without plaque) x100 | Expressed as percentage > 0 %. | Calculations from manual contour by radiologist as an imperfect(subjective) reference standard. |
| **(maximum) Wall Thickness** | Calculated by measuring the largest thickness of wall | Expressed in units of mm | Calculations from manual contour by radiologist as an imperfect(subjective) reference standard |
| **Endothelial Shear Stress (ESS)** | Tangential stress derived from the friction of flowing blood on the vascular endothelium surface and is ESS is proportional to the product of the blood viscosity and the spatial gradient of blood velocity at the wall (ESS=dv/dy). | Expressed as force/unit area ($N/m^2$ or Pascal or $dyne/cm^2$). Magnitude that ranges from 15 to 70 $dyne/cm^2$ over the cardiac cycle and yields a positive time average. | Literature ranges to establish plausibility (no truth standard applied in lieu of independent measurement modality). |

## 2.2 Rationale

We hypothesize that *in vivo* MRI examinations of patients, when suitably acquired and analyzed with the vascuCAP™ analysis software, can provide a sufficiently accurate profile of plaque characteristics to provide an objective basis for patient management.

We propose to use MRI to evaluate the extent, as well as, the structure, composition, and functional aspects of atherosclerotic plaques in >=110 patients (80 carotid patients and 30 femoral patients) scheduled to undergo an endarterectomy of the aforementioned vascular beds as part of their routine clinical care. These patients will be undergoing endarterectomies to mitigate their clinical risk or symptoms for things such as strokes, claudication, and critical limb ischemia. The endarterectomy specimens removed at surgery will allow a direct comparison between the MRI information obtained prior to the surgery, and the histopathological analyses of the arterial specimens. All patients will undergo a MRI examination within 30 days of the surgical procedure. Histological samples will be analyzed to identify neovascularization, intra-plaque hemorrhage, collagen types, necrotic core, and other hallmarks of plaque severity.

Histology and MRI will be co-registered, step-sectioning through the endarterectomy specimen using the suture-marked proximal and distal ends and anterior face as the anatomical markers. We create ground truth maps from histology of the excised specimens with the pre-operative MRI. This allows for careful mapping of the precise extent of plaque from the corresponding pathologic lesion onto the *in vivo* imaging. This will allow for careful training and testing of the image based classifiers to identify and characterize the plaque regions. Study duration, from

IRB approval through analysis and close of study is expected to require approximately 18 months, with 12 months targeted for subject enrollment and acquisition of samples, and 6 months to complete analyses and reporting.

## 2.3 Potential Risks and Benefits

### *2.3.1 Risks*

There will be no additional risk to the patients enrolling within this study as no addition procedures or imaging will be required. Standard of care clinical workup, disease assessment, procedures, and surgical treatment will occur. Risks to patient confidentiality will be mitigated by de-identifying all patient data, specimens and samples.

Although no additional MRI outside of the standard clinical workup, the 2013 American College of Radiology (ACR) Manual on Contrast Media stated the frequency of all acute adverse events after an injection of 0.1-0.2 mmol/kg of gadolinium chelate ranges from 0.07-2.4%. The vast majority of these reactions are mild, including coldness at the injection site, nausea with or without vomiting, headache, warmth or pain at the injection site, paresthesias, dizziness, and itching. Reactions resembling an “allergic” response are very unusual and vary in frequency from 0.004% to ¬0.7%. A rash hives, or urticaria are the most frequent of this group, and very rarely there may be bronchospasm. Severe, life-threatening anaphylactoid or nonallergic anaphylactic reactions are exceedingly rare (0.001% to 0.01%)

An important severe reaction to mention is nephrogenic systemic fibrosis (NSF). NSF is a debilitating condition whose onset is associated with gadolinium contrast injection. Primary seen in patients with GFR<30 and prior gadolinium exposure. Certain agents are known to have a higher association with NSF and are considered higher risk when administered to patients with renal impairment. Incidence ranges from 36.5/100,000 to 4/100,000.

Extravascular extravasation via infiltrated IV can occur. The incidence of extravascular extravasation in one series of 28,000 doses was 0.05%. Laboratory studies in animals have demonstrated that both gadopentetate dimeglumine and gadoteridol are much less toxic to the skin and subcutaneous tissues than are equal volumes of iodinated contrast media.

Standard risk associated with the appropriate surgical procedure will be discussed with the patient prior to operation at the time of operation consent. Minimal to no risk will be accrued by the patient via pathologic examination of the removed specimen.

### *2.3.2 Benefits*

A direct benefit to this patient population will likely not be encountered during this study’s duration. Benefits will be seen in future patient populations as the vascuCAP$^{TM}$ imaging technology will be better able to identify, categorize, and score problematic vascular beds.

### *2.3.3 Risk to Benefit Ratio*

The risk to this patient population is considered minimal, while the potential benefit of progressing the vascuCAP$^{TM}$ noninvasive imaging technology could potentially allow for limited use of more invasive imaging techniques, such a angiography, in future patients. Accurate noninvasive plaque characteristics and risk scoring could also lead to individualized patient management based on predicted plaque stability through vascuCAP$^{TM}$ imaging. Future accurate

plaque assessment could potentially lead to noninvasive assessment of drug, example statins, influence on individual arterial lesions.

### *2.3.4 Therapeutic Alternatives*

Therapeutic alternatives would be limited as the MRI the patients would be set to undergo would be a part of their routine clinical workup. Although for select patients, alternative imaging strategies could include ultrasound, CT/CTA, and/or invasive angiography.

# 3 OBJECTIVES

## 3.1 Study Objectives

The vascuCAP™ measurements of structural and functional features of the arterial wall will be performed in patients undergoing endarterectomy for the following objectives:

Primary:

I. Measure the bias, precision, and reproducibility of each of the functional analytes as measured by vascuCAP™ with respect to histopathologic results as ground truth, and assess their linear region and limits of quantitation.
II. Establish performance characteristics of the structural and hemodynamic analytes, where an objective truth standard is lacking.

Secondary:

III. Classify the target lesion according to whether the analytes suggest it is not a plaque, or if it is, which of the 8 AHA types best fits the analyte profile. Assess the accuracy of vascuCAP™'s classification against the histopathology classification.

## 3.2 Study Outcome Measures

Measured results will be compared in a blinded fashion with histology to assess performance of vascuCAP™-*Profiling* and build a prediction model in vascuCAP™–*Scoring* according to the following study endpoints:

I. Measure the bias, precision, and reproducibility of each of the functional analytes as measured by vascuCAP™ with respect to histopathologic results as ground truth, and assess their linear region and limits of quantitation:
   a. Plaque lipid core pool presence and volume.
   b. Plaque fibrosis volume, degree, and uniformity.
   c. Plaque inflammation burden, degree, and uniformity.
   d. Intra-plaque hemorrhage burden and confidence.
   e. Plaque vascular leak - volume, degree, and uniformity.
   f. Calcification – volume, degree, and uniformity.

II. Establish performance characteristics of the structural and hemodynamic analytes, where an objective truth standard is lacking:

   a. Determine the interchangeability of structural measurements of wall thickness, remodeling ratio and luminal narrowing and hemodynamic measurements (ESS) with an imperfect (subjective) reference standard of manual contouring by radiologists. vascuCAP™ will be evaluated under two scenarios: fully-automated vascuCAP™, and a workflow where a reader will have the opportunity to review and adjust vascuCAP™ produced results.

   b. Assess the reproducibility under different clinical sites, scanner models, and field strengths (dependent variable of interest being the interchangeability for each analyte relative to the imperfect reference standard) and with respect to ranges documented in literature according to collected co-variates.

III. Classify the target lesion according to whether the analytes suggest it is not a plaque, or if it is, which of the 8 AHA types best fits the analyte profile. Assess the accuracy of vascuCAP™'s classification against the histopathology classification.

# 4 STUDY DESIGN

A two-phase study is proposed. In the first phase, 75 surgical patients will be recruited as part of a training set; aims I-II will be evaluated with this set. In the second phase, at least a minimum of 35 and as many as 75 surgical patients will be recruited (number to be set based on the results of a statistical analysis following the first phase). Data from the second phase will be used in the definitive results to be filed in a 510(k) application to the FDA documenting the statistical performance of vascuCAP$^{TM}$'s ability to measure functional and structural analytes according to Aims I and II. Data collected in the second phase will also be used as additional training data for Aim III.

vascuCAP$^{TM}$'s performance in measuign functional analytes will be assessed using histology of specimens collected during surgery as ground truth. vascuCAP$^{TM}$'s performance in measuring structural analytes will be facilitated by comparing its automated segmentations against manual contours produced independently by two radiologists recruited for the study. The two radiologists will independently perform the manual contouring for all study patients, and one of these two radiologists will also perform the vascuCAP$^{TM}$ aided contouring after a sufficient wash-out period after the manual contouring and with appropriate blinding to their manual results.

Patients are recruited, enrolled, and analyzed with vascuCAP$^{TM}$. 75 patients which have been imaged and on which surgery was performed within 30 days of imaging will form the training set. An analysis will be performed and revisions to vascuCAP$^{TM}$ will be made, as appropriate. The sample size for the *Profiling* will be determined at this time; we denote this as $N_{profile}$. After analysis, the product configuration will be locked, and subsequent patient results are blinded. After $N_{profile}$ additional patients have been imaged and on which surgery was performed within 30 days of imaging ($N_{profile} \geq 35$).

# 5 STUDY ENROLLMENT AND WITHDRAWAL

Patients scheduled for MRA and endarterectomy at the LSU ILH Medical Center, New Orleans as well as West Jefferson Medical Center, Marrero will be recruited for the study after informed consent has been obtained. All patients will undergo a carotid or femoral artery MRI examination within 30 days of the surgical procedure. Patients will not be coerced into enrolling within the study. Patients will be reminded of their freedom to abstain from enrollment. Patients will be reminded that upon abstaining from enrollment, no objective or subjective change will/would occur in their care.

**Current Standard of Care and Deviation Thereof:**
Patients will be recruited, enrolled, and the vascular bed in question analyzed with MRI and the vascuCAP$^{TM}$ system. For a patient to be included within the appropriate phases as described previously, any vascular operation upon the vascular bed in question must take place within 30 days of the vascuCAP $^{TM}$ study. MRI for carotid and femoral arterial atherosclerotic lesions is consistent with current standard of care [7]. Carotid and femoral lesion vessel distribution and percent stenosis can be accurately assessed with MRI. Thus, preoperative imagining via MRI is sufficient to construct an appropriate operative plan for individual patients. The MRI which will be required for patients to undergo will *not* be an addition to their clinical work up had they not enrolled in the study. Therefore, no additional risk will be seen by patients outside of the standard clinical workup for their specific disease.

## 5.1 Subject Inclusion Criteria

Individuals must meet one of the following inclusion criteria in order to be eligible to participate in the study:

a. Case subjects will be patients with documented carotid atherosclerosis, scheduled for magnetic resonance angiography and subsequent elective endarterectomy with 30 days of enrollment in the study
b. Case subjects will be patients with peripheral arterial disease (PAD) with clinical symptoms, scheduled for magnetic resonance angiography and indicated endarterectomy of diseased areas of femoral arteries within 30 days of enrollment in the study

## 5.2 Subject Exclusion Criteria

Individual meeting any of the following criteria will be excluded from participation in this study:

a. Subjects with claustrophobia or inability to tolerate prior MRI studies.
b. Subjects with metal implants that are not MRI compatible (LSU detailed checklist to be attached) including: Subjects with any type of bio-implant activated by mechanical, electronic, or magnetic means (e.g. cochlear implants, pacemakers, neurostimulators, biostimulators, electronic infusion pumps, etc.). Subjects with any type of ferromagnetic bio-implant that could potentially be displaced or damaged, such as aneurysm clips, metallic skull plates, etc.
c. Subjects with a history of kidney disease or dialysis that are unable to receive intravenous gadolinium contrast material.
d. Female subjects. A urine pregnancy test will be required of all female subjects of

childbearing potential prior to inclusion in this study. Pregnant or nursing females will be excluded from the study.

e. Subjects who cannot adhere to the experimental protocol.

f. Subjects allergic to gadolinium contrast material.

## 5.3 Subject Withdrawal

Subjects are free to withdraw from participation in the study at any time upon request.

An investigator may terminate a study subject's participation in the study if:

- Any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the subject.
- The subject meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation.

## 5.4 Premature Termination or Suspension of Study

This study may be suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party. If the study is prematurely terminated or suspended, the principal investigator will promptly inform the IRB and will provide the reason(s) for suspension or termination. Circumstances that may warrant termination include, but are not limited to:

- Early attainment of statistical significance;
- Continuation of enrollment due to positive trend; and
- Determination of unexpected, significant, or unacceptable risk to subjects.
- Insufficient adherence to protocol requirements.
- Data that are not sufficiently complete and/or evaluable.
- Determination of futility.

## 5.5 Costs to Subjects

All research related procedures and/or medications will be provided at no charge to the subject and will not be billed to the insurance carrier.

# 6 STUDY SCHEDULE

## 6.1 Screening Visit (Day -28 to -1)

- Obtain and document consent from potential participant on the consent form.
- Review medical/dental history to determine eligibility based on inclusion/exclusion criteria.
- Review medications history to determine eligibility based on inclusion/exclusion criteria.
- Perform medical/dental examinations as needed to determine eligibility.
- Schedule study visits for individuals who are eligible and available for the duration of the study.
- Provide potential participants with instructions needed to prepare for first study visit.

## 6.2 Enrollment/Baseline Visit (Visit 1, Day 0)

- Review and confirm informed consent.
- Verify inclusion/exclusion criteria.
- Update any changes in demographic information, medical/dental history, medication history, alcohol and tobacco use history.
- Record results of physical and dental examinations.
- Collect blood/urine/saliva/other specimen.
- A single blood sample will be collected via antecubital venipuncture by a phlebotomist prior to MR imaging, 10-15 ml blood collected. The blood sample will be collected after verification of negative urine pregnancy test in women of childbearing potential.
- All patients will undergo a comprehensive baseline clinical assessment including evaluation of their cardiovascular risk factor profile. The specific information that will be collected is seen in the table below.

**Table 2: Clinical Assessment Parameters to be Collected**

| Demographics | Antecedent CV Hx & Risk Factors | Clinical Parameters | Current Medications | Clinical Biochemistry | Ultrasound Findings |
|---|---|---|---|---|---|
| Age-Years<br>Sex-% male<br>Race<br>Body Mass Index – $kg/m^2$<br>Alcohol use<br>Tobacco Use | Smoking<br>T2D<br>Hypertension<br>Hypercholesterolemia | Heart Rate<br>Systolic BP<br>Diastolic BP<br>Ankle-brachial index | Aspirin<br>Clopidogrel<br>Statin<br>blocker<br>ACE/ARI<br>Calcium channel blocker<br>Other anti-hypertensives<br>Oral nitrates | Total Cholesterol<br>HDL-C, LDL-C<br>VLDL<br>Triglyerides<br>Creatinine<br>BUN<br>AST<br>ALT<br>Bilirubin<br>hsCRP<br>Uric Acid<br>Salivary cortisol | Thickness of anterior and distal wall of artery with lesion.<br>Assessment of flow restriction |

## 6.3 Imaging Visit (Day 0-29)

- Subjects, meeting inclusion and exclusion criteria, will be contacted by research assistant or physician in person or by telephone to complete pre-MRI consent screening by use of the MRI safety checklist. After confirming consent to study protocol, those willing to participate will be scheduled to a 1-hour time-slot to appear at the Department of Radiology to undergo an MRI.
- At the appointed time, subjects will be met by a research assistant or physician and will be oriented. Consent will be confirmed both for the MR imaging as well as the pathology specimen (carotid endarterectomy specimen after surgery in carotid artery patients and peripheral arterial specimen in PAD patients) and the MRI safety checklist repeated. Subjects will change into hospital scrubs (provided) and remove any jewelry, watches, etc.
- The subject should be asked if he or she had a permanent coloring technique (i.e., tattooing) applied to any part of the body. This includes cosmetic applications such as eyeliner, lip-liner, lip coloring, as well as decorative designs.
- The subject should be informed of the risks associated with the site of the tattoo.
- The subject should be advised to immediately inform the MRI technologist regarding any unusual sensation felt at the site of the tattoo in association with the MR procedure.
- As a precautionary measure, a cold compress (e.g., ice bag) may be applied to the tattoo site during the MR procedure.
- Women of childbearing potential will be asked to provide a urine sample for a urine pregnancy test before each MRI study. If the test is negative, the patient may continue to participate. A positive pregnancy test will exclude further participation.
- Subjects will then be escorted by a research assistant/ physician into the MRI scan room and asked to lie on the imaging table. The patient will undergo MRI imaging of the specific arterial target, which is to include post-gadolinium contrast enhanced images. The total scan duration will be less than 60 minutes. All equipment for monitoring (ECG and respiration) and imaging is approved for human clinical use.

**Table 3: Image acquisition sequences used in the protocol**

| Contrast | T1 | T2 | TOF | Gadolinium | Diffusion | Phase Contrast |
|---|---|---|---|---|---|---|
| **Imaging** | BB | T2 weighting | MRA | DCE | DWI | Q-flow |
| **Sequence** | 2D-TSE or FAST SE | 2D-TSE or FAST SE | T1-FFE or FLASH or SPGR | T1-TFE THRIVE or VIBE or FGRE | SE-EPI | T1-FFE or FLASH or SPGR |
| **Acquisition** | Before and after contrast inf; Fat Sat | Long TR, long TE; Fat Sat; inflow suppression | Fat Sat; inflow suppression | T1 weighted; Fat Sat | B values at 0, 500, and 1000 s/mm$^2$ | Choosing in phase and out of phase TEs |

- **Carotid Artery.** All carotid MR axial image acquisitions will have a FOV of maximum 140×120mm, a slice thickness of maximum 4mm, and an acquisition matrix of at least 280×256, and a longitudinal coverage of at least 30mm. The MR imaging will be centered on the bifurcation of the symptomatic carotid arteries, defined as the arteries responsible for the neurological symptoms.
- **Femoral Artery:** The femoral artery will be scanned employing a standard leg coil placed around the thigh and conventional prospective ECG triggering. All femoral MR axial image acquisitions will have a FOV of maximum 140×120mm, a slice thickness of maximum 4mm, and an acquisition matrix of at least 280×256, and a longitudinal coverage of at least 15 cm. The MR imaging will start at the femoral bifurcation and continuing through the adductor canal.

Gadolinium intravenous injection (0.1mmol/kg) will take place in between the MRI scans while the subject is lying in the scanner. A physician will be present at all times. During the imaging procedure, subjects will be monitored physiologically by the ECG and respiration monitoring devices, and also observed by closed circuit television. Further, they will have an attached emergency call button in-hand to activate voice communication. Following completion of the imaging study, subjects will be excused and their participation in the study complete.

## 6.4 Histopathology for Patients Undergoing Surgery within 30 Days of MRI

At the time of surgical excision, a suture will be placed at the anterior aspect of the proximal end (closest to the heart) of each endarterectomy specimen and at least 1 additional suture placed on the anterior aspect of the specimen to allow for proper orientation of the specimen during embedding and sectioning of the specimen so that the sections obtained can be aligned with the MR images. After endarterectomy during surgery, the excised plaque specimens will be sent to the hospital's pathology lab to be analyzed by two board-certified pathologists. Established hospital histopathology protocols will be used for the histology processing. The specimens will be fixed in standard fashion in 10% buffered neutral formalin, decalcified, and then processed routinely to obtain paraffin blocks. Samples will be step-sectioned to obtain four (10 μm thick) sections every 2.0 mm throughout the length of the endarterectomy specimen and stained (Hematoxylin-Eosin, Masson's trichrome, Verhoeff van Gieson and Picrosirius red). The H&E and Masson's Trichrome will be used to identify morphological and structural features for categorizing according to the proposed modified AHA classification scheme [8]. Picrosirius Red Staining is used to identify demarcations of the connective tissue and the extent and type of fibrotic tissue (collagen) present in the intimal regions of the lesions [9]. The sections will be stained with Masson's trichrome (Sigma Aldrich) for cellular components [SMC-pink, red blood cells (RBC)-red], fibrous tissue (blue), fibrin and platelet-rich thrombus (deep purple). Picrosirius red (Electron Microscopy Sciences, Hatfield, PA) staining is used to identify the type of collagen fibers (type I-old fibers stain yellow-orange and type III-new fibers stain green). The Verhoeff's van Giesen stained sections will be used to identify the internal (IEL) and external elastic laminae (EEL) which delineate the boundaries of the intima and media (IEL) and the media and adventitia. The lipid core is evaluated by use of all four sections, and defined as the negative staining area that may contain inflammatory cells, collagen and elastin fibers, The presence and area of classic cholesterol clefts will also be assessed. Atherosclerotic plaques will be categorized into early (types II and III) and advanced (types IV, Va, Vc and VI) plaques according to a proposed modified American Heart Association (AHA) classification. Type VI plaques were further subdivided into VI-ruptured and VI-eroded to discriminate whether thrombi were associated with demarcation of the endothelial layer of the intima or not. Rupture was defined as a discontinuity of the endothelium resulting in a contact of the thrombus with the underlying lipid core. Erosion was identified when the thrombus is attached to an intima that lacked endothelium and an underlying myointimal region that is rich in SMC and proteoglycans. Plaques types VII (calcification) and VIII (fibrotic) are proposed as two forms of regression of

plaques that may be associated with intensive lipid lowering drug regimens [10]. Calcification and fibrosis will be identified with the H&E and Masson's Trichrome stains.

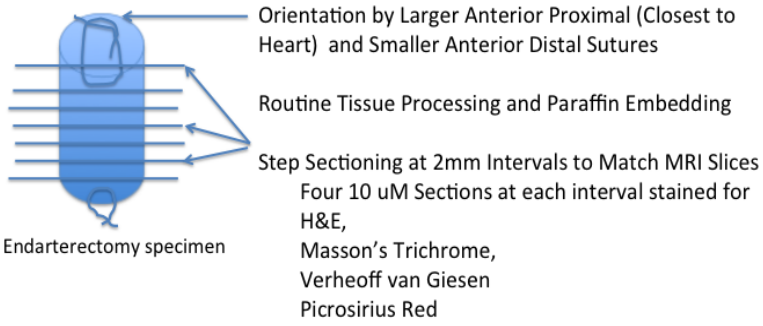


**Figure 1: Endartectomy Specimen Orientation and Process Map**

# 7 SAFETY MONITORING

The investigator will be responsible for study oversight, including monitoring safety, ensuring that the study is conducted according to the protocol and ensuring data integrity. The PI will review the data for safety concerns and data trends at regular intervals, and will promptly report to the IRB and NHLBI any Unanticipated Problem (UP), protocol deviation, or any other significant event that arises during the conduct of the study.

Safety monitoring for this study will focus on unanticipated problems involving risks to participants, including unanticipated problems that meet the definition of a serious adverse event.

## *7.1.1 Unanticipated Problems*

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to subjects or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

## *7.1.2 Serious Adverse Events*

A serious adverse event (SAE) is one that meets one or more of the following criteria:

- Results in death
- Is life-threatening (places the subject at immediate risk of death from the event as it occurred)
- Results in inpatient hospitalization or prolongation of existing hospitalization
- Results in a persistent or significant disability or incapacity
- Results in a congenital anomaly or birth defect

An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

## 7.2 Reporting Procedures

Incidents or events that meet the OHRP criteria for unanticipated problems require the creation and completion of an unanticipated problem report form. OHRP recommends that investigators include the following information when reporting an adverse event, or any other incident, experience, or outcome as an unanticipated problem to the IRB:

- Appropriate identifying information for the research protocol, such as the title, investigator's name, and the IRB project number;
- A detailed description of the adverse event, incident, experience, or outcome;
- An explanation of the basis for determining that the adverse event, incident, experience, or outcome represents an unanticipated problem;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the unanticipated problem.
- Additional information required by the IRB as listed in Section 4.7 of the LSUHSC IRB Policies and Procedures Guidebook will also be provided in a timely manner.

To satisfy the requirement for prompt reporting, unanticipated problems will be reported using the following timeline:

- Unanticipated problems that are serious adverse events will be reported to the IRB and to NHLBI within 1 week of the investigator becoming aware of the event.
- Any other unanticipated problem will be reported to the IRB and to NHLBI within 2 weeks of the investigator becoming aware of the problem.
- All unanticipated problems should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), and OHRP within one month of the IRB's receipt of the report of the problem from the investigator.

All unanticipated problems will be reported to NHLBI's centralized reporting system via Rho Product Safety:

- Product Safety Fax Line (US): 1-888-746-3293
- Product Safety Fax Line (International): 919-287-3998
- Product Safety Email: rho_productsafety@rhoworld.com

General questions about SAE reporting can be directed to the Rho Product Safety Help Line (available 8:00AM – 5:00PM Eastern Time):

- US: 1-888-746-7231
- International: 919-595-6486

# 8 STATISTICAL ANALYSIS

A two-phase study is proposed. In the first phase, 75 surgical patients will be recruited as part of a training set; aims I-III will be evaluated with this set. In the second phase, at least 35 surgical patients will be recruited (possibly more, depending on the results of the first phase). Data from the second phase data will be used in the 510(k) application (i.e. Report of the results of the performance of the functional and structural analytes) and as additional training data for Aim III.

Two radiologists will be recruited for the study. The two radiologists will independently perform the manual contouring for all study patients, and one of these two radiologists will also perform the vascuCAP™ aided contouring after a sufficient wash-out period after the manual contouring and with appropriate blinding to their manual results.

## 8.1 Aim I

The first study aim is to estimate the bias and precision of each of the following functional analytes, which will be measured for each target lesion by both vascuCAP™ and histopathology:

a. Plaque lipid
b. Plaque fibrosis
c. Plaque inflammation
d. Intra-plaque hemorrhage burden and confidence.
e. Plaque vascular leak
f. Calcification

For each of these functional analytes, three measurements will be evaluated for a total of 18 measurements per target lesion: volume in $mm^3$, and degree and uniformity both measured on a three-point ordinal scale. The same measurements will be taken via histopathology, where the volume of each analyte will be measured using the same units (i.e. $mm^3$); however, degree and uniformity are graded subjectively on histopathology by the pathologist.

We anticipate that each patient will have at least one target lesion and some patients, especially the PAD patients, will have two target lesions, on average. There will be no replicate measurements (i.e. all of the measurements are obtained objectively by the algorithm so multiple runs should not produce different results).

For volume measurements for each analyte for each site (i.e. neck and leg), we will plot the value measured by histopathology vs. the difference between the value measured by vascuCAP™ and the value measured by histolopathology. Any analyte not measurable will also be indicated on the plot using a different plotting character. The resulting plot will be inspected visually for the following:

1. Limits of vascuCAP™'s quantitation;
2. Associations between the magnitude of the histopathology measurement and bias; and
3. Associations between the magnitude of the histopathology measurements and heteroscedasticity in the vascuCAP™ measurements.

If the plots of carotid and femoral entarterectomies have similar distributions, then the data from these sites will be pooled; otherwise, separate analyses will be performed.

A linear model will be fit (for all of the data or for a subset of histopathology values, depending on the findings from the plot) where the dependent variable is the vascuCAP™ measurement (*X*) and the independent variable is the histopathology value (*Y*). Generalized estimating equations (GEEs) (to account for the fact that some patients will have more than one target lesion) will be used to estimate the linear function: $E(Y|X) = \beta_0 + \beta_1 X$. An unstructured working covariance will be used. We will construct a 95% CI for the intercept $\beta_0$ using a t-statistic as the pivotal statistic. CIs not containing 0 indicate fixed bias. We will also construct a 95% CI for the slope $\beta_1$. CIs not containing 1 indicate proportional bias [11].

The precision of the vascuCAP™ measurements will be estimated by the standard deviation: $\sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(Y_i - X_i - \bar{d})^2}$ where $\bar{d}$ is the sample mean of the differences, $\bar{d} = \frac{1}{n}\sum_{i=1}^{n}(Y_i - X_i)$ [1]. We will construct a 95% CI for the precision using bootstrap methods.

We will plot the bias profile (plot of bias of measurements for various ranges of histopathology values vs. the histopathology value) and precision profile (plot of standard deviation of vascuCAP™ measurements from patients with similar histopathology values vs. the histopathology value) as visual summaries of vascuCAP™ performance for the bias and precision components, respectively [12].

In addition to reporting these technical performance characteristics of the volume measurements, we will also report i) the coverage probability (CP) and ii) a discrimination index [11]. The CP is the probability that the absolute difference between the value measured by vascuCAP™ and the value measured by histology is less than $d_0$, i.e. $\pi = \Pr(|Y - X| < d_0)$. We will plot the CP for a range of values for $d_0$. The discrimination ability of the measurements, i.e. the ability of vascuCAP™ to discriminate between different magnitudes of histopathology values, will be measured using the area under the ROC curve for continuous measures [13]. 95% CIs for the CP and ROC area will be constructed using methods for clustered binary data [14] and clustered ROC data [15], respectively.

The volume measurements are considered the most critical because they will be used to construct a score to predict rupture risk. Thus, we considered the width of 95% confidence intervals for the bias and the between-subject variance as a function of sample size (see Tables 1 and 2). The following assumptions were made: 1) the volume measurements are normally distributed; 2) carotid artery patients contribute one target lesion each, while PAD patients contribute two target lesions each; 3) target lesions from the same patient are moderately correlated (r=0.5); 4) one-quarter of all patients will be PAD patients; 5) results from femoral and carotid target lesions can be pooled; 6) the average volume of an analyte is 25mm$^3$; and 6) the precision of the vascuCAP™ measurements is 10-20% of the volume of analyte.

From Table 4, if the SD was 15% of the mean volume, then we expect to be able to construct a 95% CI for the bias of total width of 10% with n=35. If the SD was 20%, then with n=35 we expect to construct a 95% CI of total width of 14%. Similarly, from Table 5, if the SD was 15% of the mean volume, then with n=35 we expect to be able to construct a 95% CI for the precision of total length 8%; if the SD was 20%, then we expect to be able to construct a 95% CI for the precision of total length 11%. We plan to recruit 75 patients (55 carotid artery patients and 20 PAD patients) in phase one to account for the fact that the estimates of bias and

precision may need to be reported in strata based on the magnitude of the histopathology volume and/or by anatomic location. In phase two we plan to recruit at least the minimum of 35 (25 carotid artery patients and 10 PAD patients) and possibly more depending on the results of the training set.

**Table 4: Width of 95% CIs for Bias Based on Total Sample Size (n)***

| | n=10 | n=20 | n=30 | n=40 | n=50 | n=60 |
|---|---|---|---|---|---|---|
| SD=2.5 (10%) | ±1.72 | ±1.12 | ±0.90 | ±0.77 | ±0.68 | ±0.62 |
| SD=3.75 (15%) | ±2.58 | ±1.69 | ±1.34 | ±1.15 | ±1.02 | ±0.93 |
| SD=5.0 (20%) | ±3.44 | ±2.25 | ±1.79 | ±1.54 | ±1.37 | ±1.24 |

*The effective sample size, m, is calculated as m=(3/4)n+(1/4)n(2/[1+(s-1)0.5]), where s=2. Then the half-width of the 95% CI for bias is $t_{(m-1),\frac{\alpha}{2}}\ (SD/\sqrt{m})$.

**Table 5: Estimated 95% CIs for SD Based on Total Sample Size (n)***

| | n=10 | n=20 | n=30 | n=40 | n=50 | n=60 |
|---|---|---|---|---|---|---|
| SD=2.5 | [1.80,4.77] | [1.98,3.81] | [2.08,3.51] | [2.11,3.29] | [2.16,3.20] | [2.19,3.14] |
| SD=3.75 | [2.70,7.16] | [2.97,5.71] | [3.11,5.26] | [3.17,4.94] | [3.24,4.80] | [3.29,4.71] |
| SD=5.0 | [3.60,9.54] | [3.96,7.61] | [4.15,7.02] | [4.22,6.59] | [4.31,6.40] | [4.38,6.29] |

*The effective sample size, m, is calculated as m=(3/4)n+(1/4)n(2/[1+(s-1)0.5]), where s=2. Then the 95% CI for the SD is $[\sqrt{\frac{(m-1)s^2}{\chi^2_{\frac{\alpha}{2},(m-1)}}}, \sqrt{\frac{(m-1)s^2}{\chi^2_{\left(1-\frac{\alpha}{2}\right),(m-1)}}}]$.

For degree and uniformity measurements for each analyte for each site (i.e. neck and leg), we will tabulate the histopathology value vs. the vascuCAP™ value. Any analyte not measurable will also be indicated in the table. In the distributions in the tables are similar, then the data from the two sites will be pooled.

The coverage probability (CP) will be reported which is the probability that the absolute difference between the value measured by vascuCAP™ and the value measured by histology is less than d0, i.e. $\pi = \Pr(|\mathrm{Y} - \mathrm{X}| < \mathrm{d}_0)$. We will report the CP for values for d0 of 0 and 1. 95% CIs for the CP will be constructed using methods for clustered binary data [6].

For volume, degree, and uniformity of the six functional analytes, we will estimate the reproducibility of the measures at different institutions (two sites), and under different scanner models and field strengths (1.5 and 3.0) and with respect to ranges documented in the literature according to collected patient characteristics. Multiple variable linear and logistic regression models will be built, where the dependent variable is the difference between the measured functional analyte and the true value, and the independent variables are institution, scanner model, field strengths, and patient characteristics. We will test whether the independent variables in the model are predictors of bias using F-tests, and Wald tests, respectively.

## 8.2 Aim II

The second study aim is to establish performance characteristics of each of the following structural analytes, which will be measured for each target lesion by both vascuCAP™ and two radiologists' performing manual contouring:

a. Remodeling ratio

b. Percent Stenosis

c. Maximum wall thickness

This study aim also applies similar analysis to the hemodynamic analyte endothelial shear stress since even though the computational method differs, yet it still depends on the location of contours

The vascuCAP™ measurements will be made under two scenarios: vascuCAP™ fully automated (fully automated mode) and vascuCAP™ followed by adjustments by a radiologist (semi-automated mode).

In the neck and leg, for each structural analyte, we will first visually compare vascuCAP™'s measurements to the median of the two radiologists' measurements. We will plot the radiologists' median value vs. the value measured by vascuCAP™ in each vascuCAP™ mode. Any analyte not measurable will also be indicated on the plot using a different plotting character. The resulting plot will be inspected visually for the following:

1. Limits of vascuCAP™'s quantitation;

2. Associations between the magnitude of the analyte and the magnitude of the discrepancy between vascuCAP™ and the radiologists' measurements; and

3. Associations between the magnitude of the analyte and heteroscedasticity in the vascuCAP™ measurements.

If the plots of neck and leg have similar distributions, then the data from these sites will be pooled; otherwise, separate analyses will be performed. Since the radiologists' manual contouring has both bias and measurement error, rather than assessing agreement, we will estimate the *interchangeability* of vascuCAP™'s structural measurements with the manual contouring by radiologists. The individual equivalence criterion [16, 17] will be estimated as follows:

$$\widehat{IEC} = \sqrt{\sum_{i=1}^{n}(X_i - Y_{i1})^2} - \sqrt{\sum_{i=1}^{n}(Y_{i1} - Y_{i2})^2}$$

where $X_i$ is the measurement for the i-th case by vascuCAP™, $Y_{i1}$ is the measurement for the i-th case by the first radiologist using manual contouring, and $Y_{i2}$ is the measurement for the i-th case by the second radiologist using manual contouring. IEC is a measure of the expected difference between vascuCAP™'s measurements and a radiologist's measurements with manual contouring, after adjusting for the expected difference between two radiologists' measurements with manual contouring. IEC will be estimated for both vascuCAP™ fully-

automated and vascuCAP™ semi-automated. 95% bootstrap CIs will be constructed for the IEC.

For each of the structural analytes, we will estimate the reproducibility of the measures for different factors: different institutions, different scanner models, different field strengths, and with respect to ranges documented in the literature according to collected patient characteristics. We will estimate the IEC for these different factors, and we will construct CIs for the difference between the IECs using bootstrap simulations.

## 8.3 Aim III

The vascuCAP™ measurements will be used to classify the target lesions according to whether the analytes suggest it is not a plaque, or if it is, which of the 8 AHA types best fits the analyte profile. The data from the first phase will be used to build the classifier, and the data from second phase will be used as a first assessment of the accuracy of the classifier. This is considered a pilot study for the classifier and will not be included in the *Profiling* product release until sufficient statistical significance for all outputs is demonstrated.

With the limited sample size for the assessment of the classifier, we will not attempt to model the results. Rather, we will simply report the 9x9 table of AHA types by histopathology vs. AHA types by vascuCAP™'s classifier. In addition, for each of the AHA types according to histopathology, we will report:

1. the frequency with which vascuCAP™'s classification agrees with histopathology,

2. the frequency with which vascuCAP™'s classification is within an acceptable range (see Table 6), and

3. the frequency with which vascuCAP™'s classification is not within an acceptable range.

**Table 6: Penalty Matrix for Evaluating vascuCAP™ AHA Plaque Type Classifier**

| | Truth | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Classification* | *0* | *1* | *2* | *3* | *4* | *5* | *6* | *7* | *8* |
| 0 | A | | | | | | | | |
| 1 | A | A | | | | | | | |
| 2 | NA | A | A | | | | | | |
| 3 | NA | NA | A | A | | | | | |
| 4 | NA | NA | NA | A | A | | | | |
| 5 | NA | NA | NA | NA | NA | A | | | |
| 6 | NA | NA | NA | NA | NA | NA | A | | |
| 7 | NA | NA | NA | NA | NA | NA | NA | A | |
| 8 | NA | NA | NA | NA | NA | NA | NA | NA | A |

A=acceptable
NA=not acceptable

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