Appendix

Carotid Ultrasound Training Manual

Research Neurosonology Laboratory / Ultrasound Reading Center (URC):

Carotid Ultrasound Imaging Analysis Core Laboratory

Clinical Translational Research Division (CRTD)

Department of Neurology, Miller School of Medicine

University of Miami, Miami, FL

Director: Tatjana Rundek, MD PhD

Chief Research Sonologist: Digna Cabral, RVT

List of Abbreviations

ANT	Anterior
BIF	Bifurcation
CCA	Common carotid artery
CERT	Certification
CIMT	Carotid intima-media thickness
GSM	Grey-Sale Median
ICA	Internal carotid artery
ID	Identification
LA	Left arm
LAT	Lateral
LBIFFW	Left bifurcation far wall
LBIFNW	Left bifurcation near wall
LCCANWFW	Left common carotid artery near wall and far wall
LICAFW	Left internal carotid far wall
LICANW	Left internal carotid artery near wall
LT	Left
OAI	Optimal anatomical interrogation angle
POST	Posterior
PW	Pulse wave
QA	Quality assurance
QC	Quality control
RA	Right arm
RBIFFW	Right bifurcation far wall
RBIFNW	Right bifurcation near wall
RCCANWFW	Right common carotid artery near wall and far wall
RICAFW	Right internal carotid far wall
RICANW	Right internal carotid artery near wall
RT	Right
TFD	Tip of flow divider
URC	Ultrasound Reading Center

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Carotid Ultrasound Training and Certification Protocol

1. Introduction to Carotid Ultrasound Training

All sonographers must participate in a uniform training and certification program coordinated by the Carotid Ultrasound Imaging Analysis Core Laboratory at the Clinical Translational Research Division (CRTD) in the Department of Neurology of the Miller School of Medicine, University of Miami (or Ultrasound Reading Center, URC). All procedures, documentation, and certification for the sonographers will be provided by URC.

1.1 Personnel

Sonographers at the 4 imaging centers will be required to perform the ultrasound scanning. All sonographers must complete a uniform training program conducted at URC. Sonographers are certified upon satisfactory documented completion of the training and certification program. Sonographers should equally share the scanning responsibilities during the study to maintain a high skill level and certification requirements. Due to this reason, the concept of a "primary" and a "back-up" sonographer in longitudinal studies of this type is inappropriate since the skills must be practiced on a regular basis by each sonographer to maintain a high skill level.

1.2 Instrumentation

The HCHS-SOL Imaging Centers will be equipped with Terason uSmart 3300 NexGen Ultrasound system with a 15L4A MHz uSmart Linear Array Transducer with system or probe range. Other ultrasound systems can be used if they have available an appropriate 10-15MHz linear array transducer and a pre-set vascular protocol. This ultrasound system should be capable of recording and storing multiple digital clips of ultrasound images per participant in digital format. Participant identifiers and demographic information can be entered in the ultrasound system and embedded in the header of the image files.

1.3 Sonographer Certification

The sonographer certification requirements consist of submitting a total of up to 5 good quality protocol scans on volunteers. We recommend that these 5 scans are based on volunteers in the age group of the study population. These scans are reviewed and evaluated by URC staff and must demonstrate a high level of proficiency in the carotid ultrasound protocol. Each sonographer after completion of the scan and the log-in sheet.

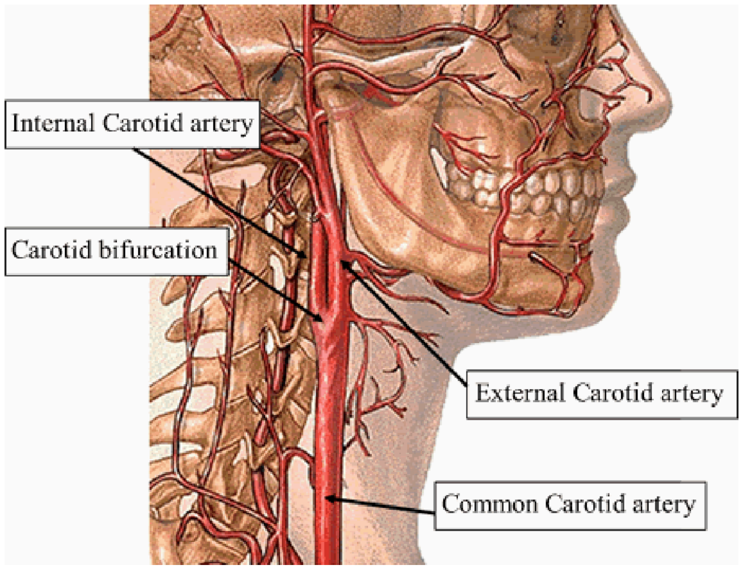
The staff of the URC will carefully evaluate the scans. The observations and comments are returned by E-mail within five working days after receipt of the ultrasound images from each sonographer to indicate the quality of performance and suggestions for improvement. The expectation is that at least 3 consecutive scans (graded as level A) are needed for a sonographer to demonstrate a high level of proficiency in the performance of the protocol and receive initial certification. Experienced sonographers, i.e., those who have worked with a similar ultrasound protocol in previous studies will also need to provide these protocol scans to the URC scans in order to be certified.

1.4 Performance and Maintenance of Equipment

It is primary responsibility of the sonographers and readers to assure that the ultrasound of reading equipment is performing optimally prior to each exam or reading. Routine preventive maintenance must be provided at the interval recommended by the manufacturers. Any service-related problems that could reduce data quality and performance during the examination should be documented in a service log and rapidly resolved by the equipment manufacturers. All upgrades or changes to the ultrasound system hardware and software must be approved in advance in writing by the URC and study sponsor. In addition, documentation must be made in the service log. Modification of the scanning or reading hardware and software can only be made during course of the study with prior written approval of the URC and sponsor to maintain scan standardization throughout the study.

1.5 Introduction to the Extracranial Carotid Artery Anatomy

The carotid arteries are the two major arteries in the neck. The right common carotid artery (CCA) originates from the innominate artery on the right and the left common carotid artery originates directly from the aortic arch. Each common carotid artery ascends in the neck laterally. Each of the two common carotid arteries bifurcates into the external (ECA) and internal carotid (ICA) artery. The external carotid arteries are more superficial with numerous arterial branches that supply the neck and face. ECA diameter is usually smaller than the ICA diameter. ECA usually runs deeply within the neck. ICA has no branches in the neck and ascends into the brain via the foramen lacerum calvarium.



1.6 Carotid Segments

The primary objective of the carotid ultrasound scanning protocol is to acquire high quality standardized longitudinal B-mode images in 12 well defined arterial wall segments in both, the right and the left carotid arteries. Each carotid artery has been divided into 3 segments, the common carotid, bifurcation and internal carotid segment (CCA, BIF and ICA) with 6 walls (near wall and far wall of each segment) as show in Figure 1. The three segments are defined as: the near wall and far wall of the arterial segment extending from 10 mm to 30mm into the distal common carotid artery (CCA); the near wall and far wall of the carotid bifurcation beginning of dilatation or 10mm below the tip of the flow divider (TFD); and the near wall and far wall of the 10mm of the internal carotid artery (ICA) distal of the tip of the flow divider.

Since the specific internal anatomy of an individual subject is variable, the near walls and far walls may correspond to different anatomical sites.

Carotid Segments

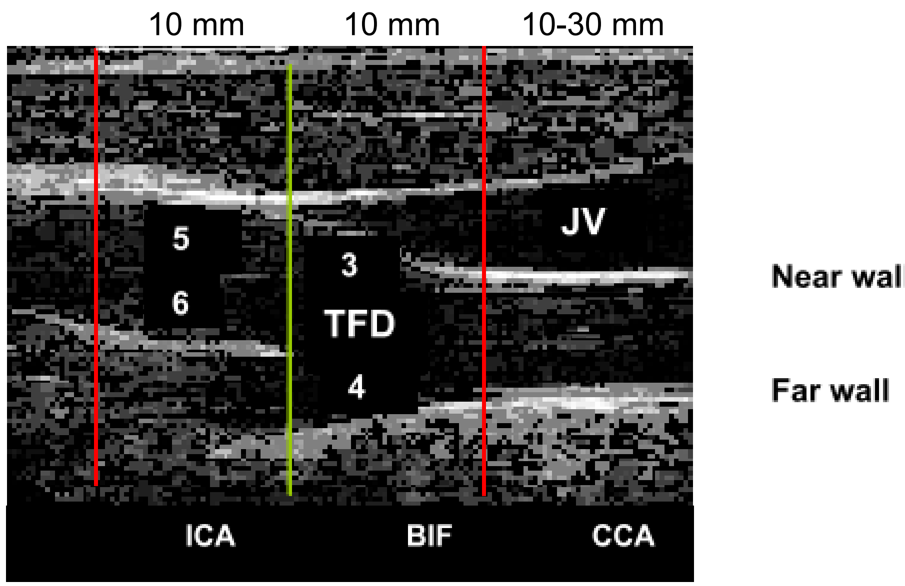


Figure 1. The 3 carotid segments and 6 walls

1.7 Carotid Disease found and exclusion base on Carotid ultrasound guidelines

This research protocol has been developed to meet specific research objectives in the study and the protocol differs significantly from the routine clinical examination of the carotid arteries performed for clinical purposes. If, as a result of the performance of this research protocol, severe and/or extensive carotid artery disease (e.g., stenosis over 50%) is discovered in a research subject, this information is rapidly communicated by the sonographer to the clinical site principal investigator or a designated co-investigator. A more detailed medical history may be obtained on this subject and the extent of follow-up will be determined by the local clinical site principal investigator. This process is called ALERT and it is detailed in a separated document.

If the imaging circumstances are very poor with limited boundary visualization or the protocol cannot be followed due to anatomical constraints, sonographer must make a note in the report and follow the scanning protocol to the sonographer’s best abilities and as much as possible.

Ultrasound Scanning Protocol

2. Participant information

Correct participant demographic information is placed on the ultrasound system image screen prior to the beginning of each study. This includes Imaging Center identification number, subject number ID and Initials, visit number, date of birth (dd-mon-yyyy or mon-dd-yyyy), and sonographer ID. In addition, standard equipment setting provided during the training specifying maximum depth, frame rate, dynamic range, numbers and position of focal zones, and edge detection. Image persistence must be used in the acquisition of all images. The sonographer scans from the head of the examination table.

2.1 Exploratory Scan

The exploratory of the right and left side of the neck, consists of placing the participant in a supine position on the examination table with the head towards the sonographer during the carotid artery examination in a comfortable position that allows head rotation to either side. The participant head is rotated away from the side being scanned. Explain to the participant in a professional mater the importance of not moving during the study. A pillow is place under the participant's knee to support the lower back if needed for the comfort. A towel or a low pillow may also be place under the head of the participant.

The participant is required to rest a few minutes before proceeding with the examination to establish a stable physiologic state prior to beginning of the examination. The sonographer performs the right-side scan holding the probe in the right hand and the left side holding the probe in the left hand. When the sonographer selects each specific image sequence for measurement, annotation on screen should be acquire in the initial transverse (short-axis) and longitudinal scan image performed by the sonographer prior to the beginning of the recording of the images, of the three segments of interest: the common carotid artery

(CCA), the bifurcation (BIF), and the internal carotid artery (ICA), from the proximal common carotid artery up through the bulb into the internal and back down into the common carotid artery. No external carotid (ECA) IMT images are acquired. This will enable the sonographer to become familiar with the participant's anatomy and to help to distinguish echoes that represent plaques from the artifacts. The quality of the ultrasonic images is carefully examined by the sonographer to determine if the six arterial wall boundaries are well visualized within the specific arterial segments that will be scanned ether from an anterior, lateral or posterior prospective angle of the transducer position. The internal jugular vein (JV) could be used as an acoustic window and a positioning landmark during the scanning protocol.

2.2 Transverse (short-axis) Exploratory Scan

The transverse (short-axis) scan of the right and left common carotid artery is performed beginning at the clavicle or proximal portion of the vessel, with the patient's head position 45 degrees to the contra-lateral side of the transducer or probe. The transduce is kept over the jugular vein, so that the vein is stacked on top of the artery using it as an acoustic window. The transducer is slowly moved toward the mandible until the lumen area increases with the appearance of the carotid bifurcation (also the lumen changes shape from nearly circular to elliptical), and finally the internal and external carotid arteries are visualized. Unusual anatomic features or possible plaques may be observed during this scan at any segment. A properly performed transverse scan is show in the Figure 2. and 3.

Note: This must be done on both sides of the neck (right and left).

Transverse (short-axis) Exploratory Scan

Figure 2. Example of the scan position

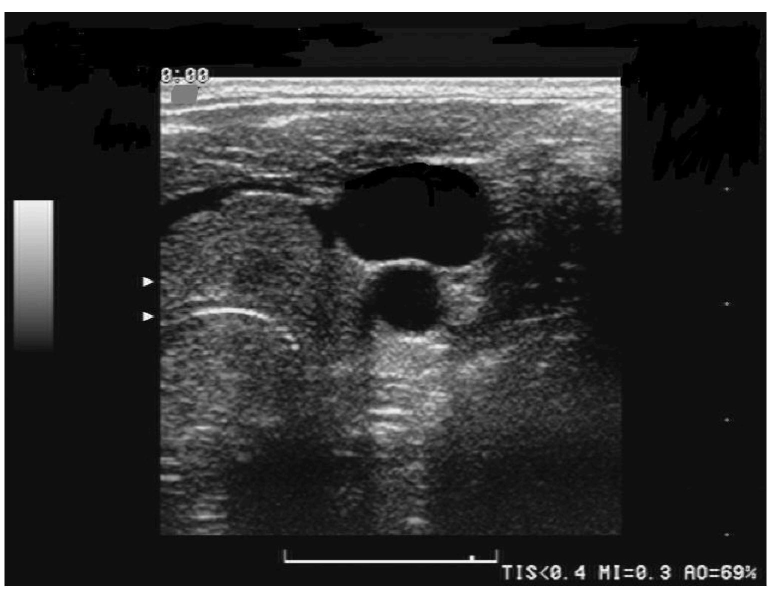


Figure 3. Transverse (short axis) Exploratory Scan

2.3 Longitudinal (long-axis) Exploratory Scan

The longitudinal (long-axis) scan of the right and left common carotid artery is performed beginning at the clavicle or proximal portion of the vessel, with the patient's head position 45 degrees to the contra-lateral side of the transducer or probe. The transducer is slowly moved toward the mandible until the lumen area increases with the appearance of the carotid bifurcation and finally the internal and external carotid arteries are visualized. Unusual anatomic features or possible plaques may be observed during this scan at any segment. A properly performed longitudinal scan is show in the Figure 4.
Note: This must be done on both sides of the neck (right and left). The sonographer exploration of the participant carotid anatomy will define the determinate angle (anterior-oblique, lateral, or posterior-oblique). The point of maximal wall thickness should be centered in the middle of the screen for each image. The sonographer should adjust the probe to maximize the wall interfaces in each projection of the IMT were best visualized to start acquiring the images that will be read by the URC.

The anterior-oblique, the lateral and the posterior-oblique scanning angles are defined as follows:

<u>Anterior-oblique</u>: the arch on the surface of the neck from the midline (trachea) to 55° to a line drawn from the mid-trachea to the center of the back of the neck.

<u>Lateral</u>: from 55° to the perpendicular to 100° (hence 45°). The sternocleidomastoid muscle can be palpated beneath this portion of the skin's surface.

<u>Posterior-oblique</u>: the arch from 100° to the perpendicular to 145°. The probe almost always lies just behind the posterior margin of the sternocleidomastoid muscle.

After this exploratory scanning, select the optimal scanning angle for IMT/plaque acquisition. Optimal scanning angle is defined by optimal visualization of IMT/plaque.

Longitudinal (long-axis) Exploratory Scan

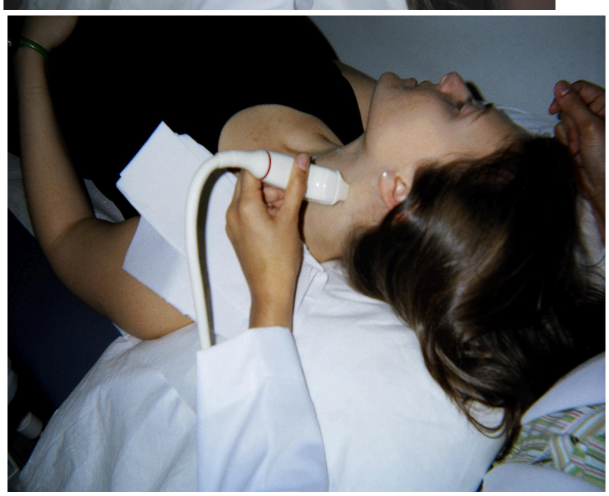


Figure 4. Example of the scan position

2.4 Satisfactory/Optimal Images

The criteria for optimal B-mode ultrasound IMT images of the carotid arteries are defined as the optimize adjusted gain setting to visualize a symmetrical brightness on long the axis view of arterial interfaces of the near and far wall.

1. Near wall-arterial wall nearest the probe
2. Far wall-arterial wall furthest from probe

The area of interest will be centered in the middle of the image and the probe will be aligned strictly perpendicular to the ultrasound beam to show as much of the vessel as possible. For optimal image quality set the frame rate to 25 Hz (>15 Hz) to facilitate edge detection. The sonographer should optimize the visualization of the interfaces by adjusting the gain settings, beam steering and transducer placement. It is expected that the lumen of a good carotid artery IMT ultrasound study will contain a significant amount of artifact, keeping the gain setting to approximately 60db higher than a typical clinical study will help to eliminate intraluminal artifacts and clearly visualize the intima-media lining or boundaries. The intima-media lining appears as two parallel echogenic lines separated by a hypoechoic space in longitudinal views of the carotid arteries. The artery walls are usually best observed in the common carotid artery where the vessel course is parallel to the skin surface and is thus a target, which is set at a right angle to the ultrasound beam.

The first echo along the far wall is derived from the lumen-intima interface and the second, normally brighter, echo along the far wall originates from the media-adventitia interface. Between these interfaces lies the media, which appears as an echo lucent zone. The distance between the first two lines corresponds to the combined thickness of the intima and media. Because of its collagen content the adventitia is quite echogenic and appears as a bright zone highlighted along its inner margin by the media and in most instances is echo lucent.

It is more difficult to image the interfaces when the near and far walls of the vessels are curvy-linear and not at right angles to the ultrasound beam. In the carotid bulb, where the walls curve, only short wall segments may be seen on any single frame. This same phenomenon is observed in the proximal portion of the internal carotid artery when the walls are not parallel and difficult to visualize. Other causes for loss of wall interfaces that are not related to scanning technique are the presence of plaques (or the presence of fat or blood) in the arterial wall. The interfaces can also be seen along the near wall, but the lines may be disrupted and the echoes weaker because the ultrasound beam is passing from solid tissue to fluid. At times, it is not possible to maximize both the near wall and far wall interfaces on the same image frame.

2.5 Optimal Anatomical Interrogation Angle (OAI)

Using the knowledge acquired of the relative anatomical orientation of the internal and external carotid arteries during the transverse scan, the optimal anatomical interrogation (OAI) angle which best displays the tip of the flow divider and the "Y" appearance of the two arteries in a single longitudinal image can be determined as it shown in Figure 5. This angle provides information about the carotid anatomy. If both extreme anterior and extreme posterior interrogation angles can provide an OAI view, the anterior angle is preferred. One way to obtain the OAI is to position both vessels on top of each other in the transverse short-axis scan and then turn the transducer longitudinally by a 90 degrees rotation to demonstrate the "Y" image appearance of the carotid arteries.

Optimal Anatomical Interrogation Angle (OAI)

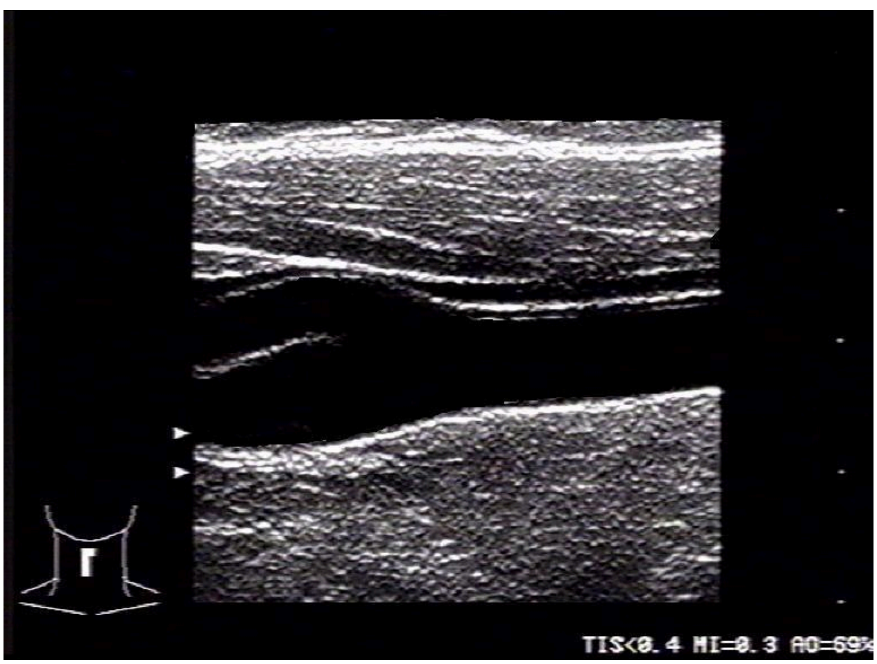


Figure 5. Longitudinal image of the carotid artery

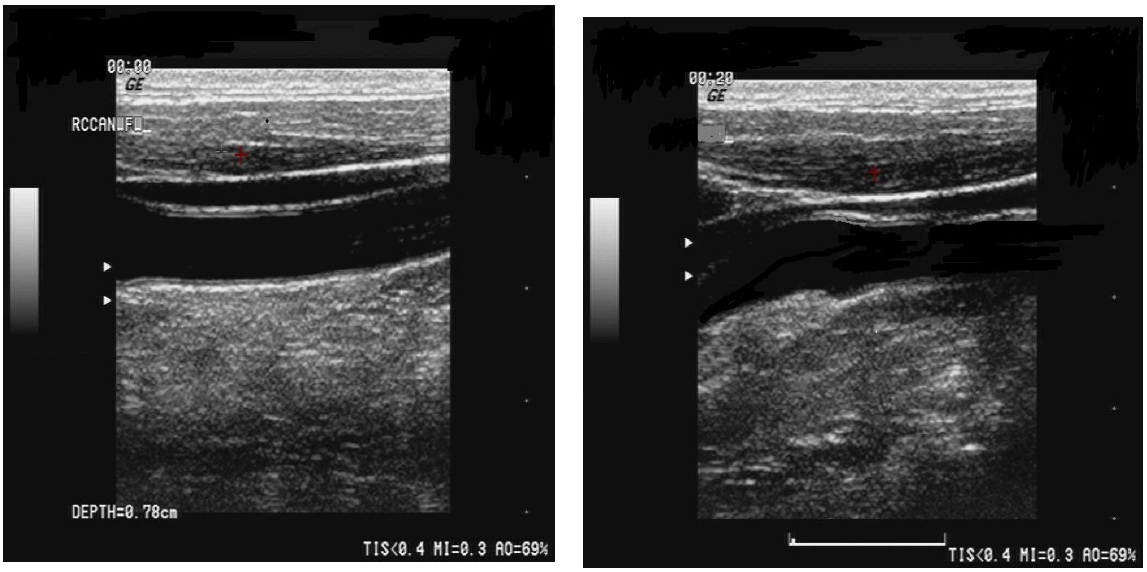
After saving the participant demographics and the optimal image stored, the following image will show the angle, which best displays the tip of the flow divider (TFD) and the “Y” image as shown in Figure 5.

3. **Preliminary CIMT Protocol Scans**

Selection of the 12 CIMT segments

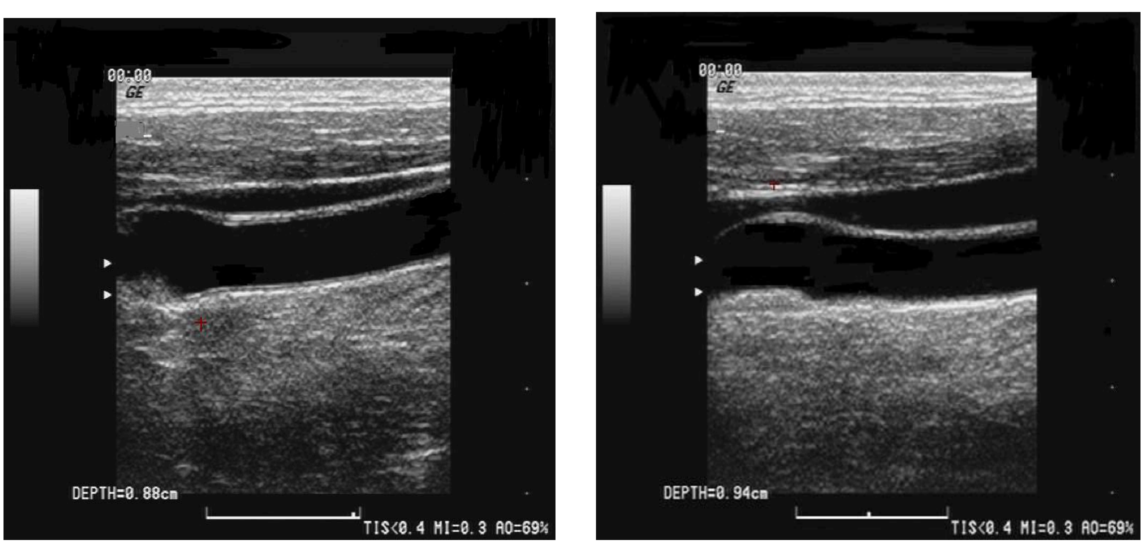
Intima Medial Thickness (IMT) is the first morphological abnormalities of arterial walls can be visualized by B-mode ultrasound. This noninvasive technique provides one of the best methods for the detection of early stages of atherosclerotic disease with high-resolution capacity, the arterial wall can be visualized, and the evaluation of blood flow velocities (BFV) obtained with the use of the pulse-wave Doppler. [1]*Carotid IMT* scanning is done on the participant’s right and then left sides of the neck. Text annotation and a caliper are used to identify the angle and IMT vessel wall under interrogation as shown in Figure 7a-7b. For the right CCA write the annotation, **RCCANWFW** and for the left, **LCCANWFW**. The caliper is always placed on top of the specific segment. For the right BIF write the annotation **RBIFNW** or **LBIFNW** for the left (Top = near wall) or **RBIFFW** or **LBIFFW** (bottom = far wall) and the same for the RICA or LICA NW or FW (**RICANW**, **RICAFW, LICANW, LICAFW**). Selections of the caliper position is used to indicate on which segment the sonographer attention is focused (Top = near wall and bottom = far wall). When a near wall is optimized, the placement of the single focus on the ultrasound system is positioned at the depth of the near wall. If a far wall is being optimized, the focus is positioned at the level of the far wall. The frozen images are saved from 6 defined segments of both sides of the neck, with a total of 12 IMT segments. No external carotid IMT images are recorded.

Selection of the 12 CIMT segments



A **B**

Fig 7a. Example of selections of IMT, caliper is placed to confirm the segment of interest.



C **D**

Fig 7b. Example of selections of IMT, caliper is placed to confirm the segment of interest.

A. CCA near and far wall: **RT/LT CCANWFW**
B. BIF near wall: **RT/LT BIFNW**
C. BIF far wall: **RT/LT BIFFW**
D. ICA near and far wall: **RT/LT ICAFNW-ICAFFW**

3.1 Carotid Plaque Protocol Scans

The location, extent, and characteristics of atherosclerotic plaque in the common carotid artery (CCA) and internal carotid artery (ICA) should be documented with gray-scale imaging. [3] The vessels should be imaged as completely as possible, with caudal angulations of the transducer in the supraclavicular region and cephalic angulation at the level of the mandible.

All plaques should be separately scanned using an optimized angle and acquired. The optimal image of plaque at its highest prominence in longitudinal axes and cross-sectional should be frozen and recorded.
Annotate:
Plaque location (e.g., RICANW – Right ICA Near Wall)

After imaging of each plaque perform Color Doppler. Color Doppler imaging should be performed to detect areas of abnormal blood flow (stenosis) that require Doppler spectral analysis.

Pulsed wave (PW) Doppler spectral analysis should be performed at the site of plaque: Proximal, Distal and in the area of the highest plaque prominence. Freeze each image at the pick systolic velocity and record the image.

As atherosclerotic lesions and plaques is visualized, as shown in Figure 6; there is an increased error of estimating anatomic abnormalities because acoustical shadowing is often present with large lesions that limits the acquisition of various segments of the vessel. Doppler measurements however, become progressively more accurate with increasing levels of disease. Shadowing is often present with calcified or very large plaques, Figure 7.

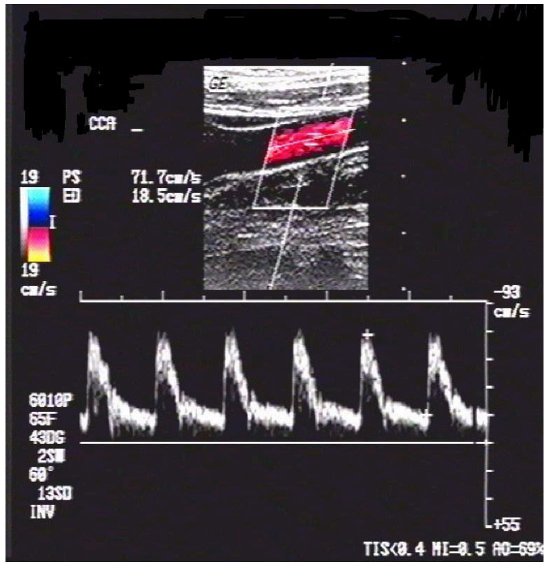
Figure 6. Example of 'soft' plaque

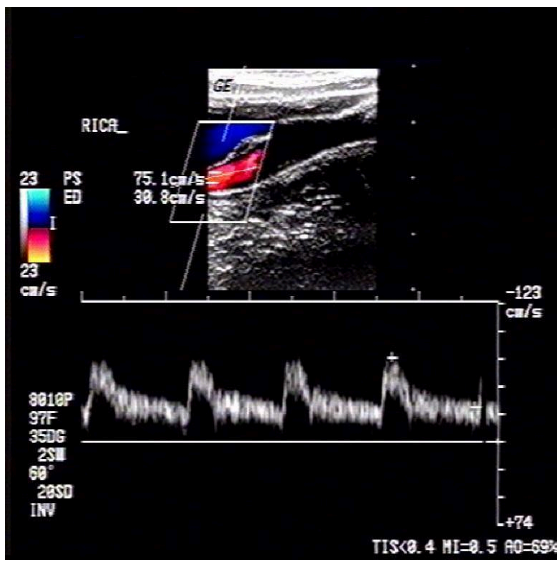
Figure 7. Example of 'mixed' plaque with calcifications and acoustic shadowing

4. Doppler Examination

After acquiring B-mode imaging, Doppler will be used for measuring blood flow velocities in examination performed on the right and left common carotid, (CCA) internal (ICA) and external carotid arteries (ECA) for quick assessment of general extracranial hemodynamic status.

A Color and pulse-wave Doppler signals at 2mm Doppler sample gate should be placed in the center of the vessel. Starting with the right distal common carotid artery (CCA), the pulse-wave Doppler, measurement is done at a depth of 4cm; angle correction must not exceed 60 degrees. A frozen image including the measurements of the peak systolic velocity (PSV) at the point of maximum flow acceleration and the end diastolic velocity (EDV); measurements should be saved. The ultrasound system will provide automatically PSV, EDS and the mean velocity. The results of the Pulse-wave Doppler's following the longitudinal scan is used to store data, help an anatomical orientation, vessel identification, and determine percent stenosis. Repeat this step for the proximal internal carotid artery (ICA) and finally the proximal external carotid artery (ECA) as shown in Figure 8.





Color Pulse-wave Doppler of Distal CCA

Color Pulse-wave Doppler of Proximal ICA

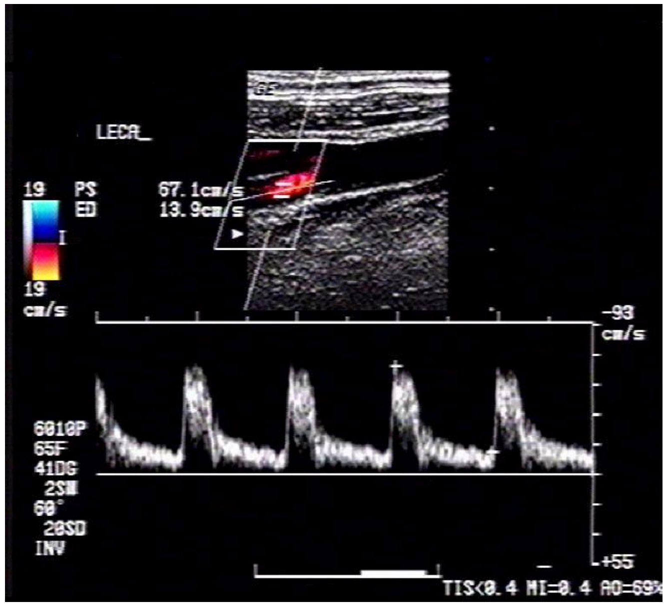


Figure 8. Color and Pulse-wave Doppler of Proximal ECA

4.1 The ALERT Procedure Response to Carotid Stenosis

Some participants will have significant severe and/or extensive carotid artery disease or carotid stenosis, which are discovered possibly for the first time during the examination at ether the common carotid, the bulb or internal carotid artery. A peak systolic pulse-wave Doppler stenosis of 50% or greater indicated by a pulse-wave Doppler measurement of peak systole of 180 cm/s (PSV) in the common carotid, bulb or internal carotid arteries, should be double check by repeating the Doppler measurement. [2] All plaques should be separately scanned using an optimized angle and acquired. Under no circumstances should this impression be conveyed either directly or indirectly to the participant by the sonographer. <u>After the participant has left the scanning area the sonographer should reported by the sonographer to the clinic coordinator and/or clinical sites principal investigator immediately (ALERT)</u>. The local clinical site principal investigator, who will determinate whether the participant will need necessary appropriate referrals for clinical evaluations as appropriate. The responsibility of the participant’s health care is completely with the field center. Whenever a participant presents with what the sonographer suspects is a problem, do not wait for confirmation from the Ultrasound Reading Center. The scan will not be reviewed for several weeks. In some cases, there may not be a Doppler value because the sonographer was unable to produce a Doppler signal; in these cases, the sonographer must make a comment to the principal investigator (PI). When Doppler values are nonexistent and the images are unclear, the reader must rely on comments from the sonographer to determine the degree of stenosis, Figure 9.

Pulse-wave Doppler stenosis

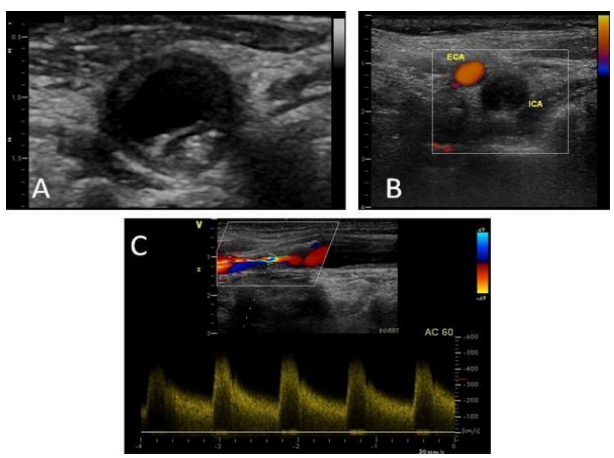


Figure 9. Color and Pulse-wave Doppler of stenosis of ICA

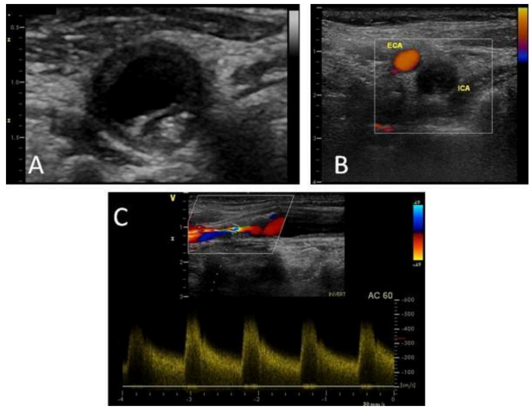


Figure 9a. Transverse view image of ICA with Plaque formation

4.2 Percent of Stenosis

The percent stenosis is determined according to the following Doppler velocity criteria: Recommended Modification of the SRU Consensus Conference Criteria for Internal Carotid Artery Stenosis for Implementation in IAC-Accredited Vascular Laboratories.

DIAGNOSTIC DOPPLER CRITERIA FOR CAROTID STENOSIS

Degree of Stenosis, %	Primary Parameters		Additional Parameters	
	ICA PSV, cm/sec	Plaque Estimate, %	ICA/CCA PSV Ratio	ICA EDV, cm/sec
Normal	<180	None	<2.0	<40
<50	<180	<50	<2.0	<40
50-69%	180–230	> 50	2.0–4.0	40–100
> 70 but less than near occlusion	>230	> 50	>4.0	>100
Near occlusion	High, low, or undetectable	Visible	Variable	Variable
Total occlusion	Undetectable	Visible, no detectable lumen	Not applicable	Not applica

*Plaque estimate (diameter reduction) with grayscale and color Doppler US.

References:

1. https://www.intersocietal.org/vascular/forms/IACCarotidCriteriaWhitePaper1-2014.pdf

2. Grant EG, Benson CB, Moneta GL, Alexandrov AV, Baker JD, Bluth EI, et al. Carotid artery stenosis: grayscale and Doppler US diagnosis – Society of Radiologists in Ultrasound Consensus Conference. Radiology 2003;229:340-6.

3. Gornik HL, Rundek T. Gardener H, et al. Optimization of duplex velocity criteria for diagnosis of internal carotid artery (ICA) stenosis: A report of the Intersocietal Accreditation Commission (IAC) Vascular Testing Division Carotid Diagnostic Criteria Committee. Vasc Med. 2021;26(5):515-5.

4.3 Plaque Morphology

Adequately documentation of the details of plaques is an important component of carotid ultrasound. Plaque can be simply characterized as homogeneous or heterogeneous.[4] Homogeneous plaques may be fibrous (soft) or calcified (hard) and have a uniform internal architecture with a smooth surface contour (Figure 6). Heterogeneous, calcify plaques and ulcerated plaques are unstable with the potential for embolic transient ischemic attacks and stroke (Figure 7). [5] [6] These plaques have lower calcium content but larger amounts of intraplaque hemorrhage and lipid content, which make them appear hypoechoic. Plaques associated with amaurosis fugax are more hypoechoic than plaques causing transient ischemic attacks or strokes. [7]Hypoechoic plaques are more likely to be symptomatic than hyperechoic ones.

4.4 Scanning Limitations

Physical challenges such as a short muscular neck, a high carotid bifurcation, tortuous vessels, calcified and shadowing plaques, surgical sutures, postoperative hematoma or bandages, inability to lie flat due to respiratory or cardiac disease or to rotate the head in patients with arthritis and cervical spine issues, and uncooperative patients may limit the results of carotid ultrasound examination.

In a presence of any of these limitations, the images may be poor. If you account any of these circumstances, please make sure to write a comment in a log sheet and explain the difficulties of scanning.

Ultrasound Reading Protocol

5. Ultrasound Image Reading

5.1 Acquisition of B-mode Image Measurement Data

The data from each of the twelve predefined arterial segments (CCA, BIF and ICA), that are required to compute the primary and secondary outcome parameters are obtained using the standardized procedure. The image sequences are obtained from an interrogation angle in each segment correctly selected. The reader performs the measurements for all segments in an identical manner analyzing each segment outline the maximum CIMT within the near wall and/or far wall boundaries and Plaque. We refer to this variable as the mean and the maximum "MeanMax" CIMT and Plaque.[6] The CIMT and plaque are traced and measured, computing the mean and maximum IMT and Plaque.

5.2 Carotid IMT Reading Protocol

Measurements are performed off-line using automated computerized edge tracking software *M'Ath* (Intelligence in Medical Technologies, Paris, France) from the recorded ultrasound images (Figure 10). In addition to CIMT, carotid plaque size (thickness and area) and echodensity is also be measured. This method is more precise in assessment of the plaque size than semi-manual method and therefore will further reduce the variance of the measurements.

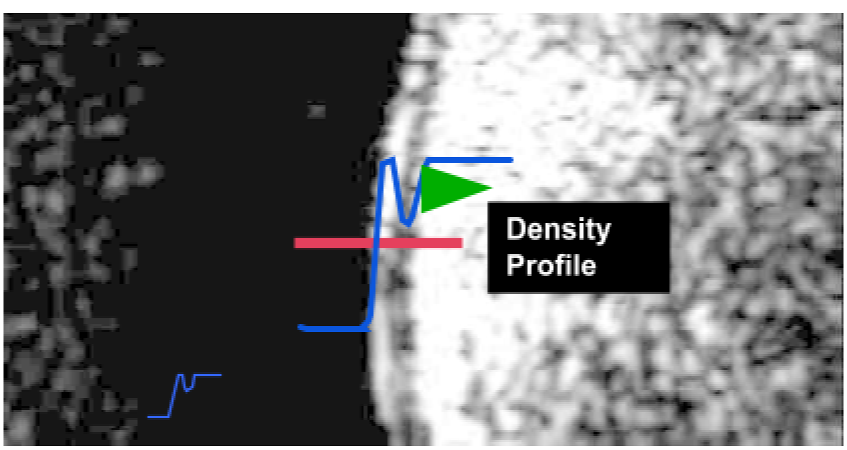
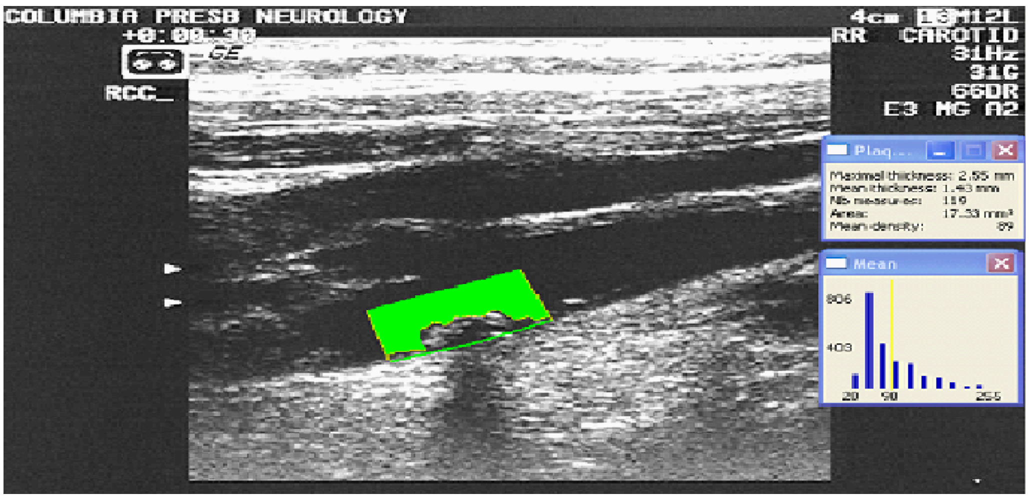


Figure 10. M'Ath measurements

The automized computer assisted program *M'Ath* tracks the near and the far arterial wall boundaries (or plaque boundaries) where reflecting signal from the carotid lumen ("reference") serves as a guide to the edge detection analyses. The program automatically searches in the vicinity of reference for the wall boundaries using an intensity gradient detection algorithm (see Figure). Application of the computerized multiframe image processing method to all the processable frames generates a sequence of carotid plaque measurements.

5.3 Carotid Plaque Reading Protocol

During scanning all carotid segments are examined for a presence of atherosclerotic plaque defined as a focal wall thickening or protrusion in the lumen more than 50% greater than surrounding wall thickness.



In addition to plaque thickness (mm) and plaque area (mm^2), plaque density expressed as the gray-scale median (GSM) index are measured by M'Ath. For each subject, the number of plaques, plaque location, plaque thickness, area and GSM index will be assessed, digitized, measured, and stored in data file. In addition, each longitudinal plaque image and degree of stenosis is corroborated against axial images to assure accurate plaque imaging in order to reduce overestimation or underestimation of plaque size. For the GSM analyses, the overall brightness of the plaque areas in the digital images is assessed by the median of the frequency distribution of tones of pixels.[4] Images are normalized with linear scaling, so that the GSM of blood is 0-5 tones, and that of the adventitia. By this image alteration, we counteract the effect of the different degrees of sonographic gain on plaque echogenicity. [9] GSM is expressed for each plaque and analyzed as a continuous variable and as categorical variable 2618 according to the quartiles of GSM distribution.

5.4 Total Carotid IMT Calculation

The mean and maximum intima-media thickness of the near and the far walls of both right and left common carotid, carotid bifurcation, and internal carotid arteries are measured in the plaque-free segments.

The total IMT is calculated as the mean (or the max) of the near and the far wall of 12 carotid segments (6 on each side of the neck).

The max, mean, and minimum lumen and the vessel diameter (width) of the carotid arteries are also determined by M'Ath.

In addition, readers will record the presence of plaques, their location, measure their thickness (the mean and the max) and area, determine acoustic shadowing, and measure plaque ultrasonic densitometry (the GSM index).

Quality Control and Quality Assurance Protocol

6. Quality Control/Quality Assurance Procedures

Throughout the study, the Ultrasound Reading Center continuously monitors the quality of the images submitted from the routine studies and the outcome variable data obtained from the within-sonographers and both within- and between-reader QC studies. Should low quality studies related to scanning technique be observed and identified, these observations are immediately reported to the sonographers and are remedied in consultation with the sonographer. Sonographers and readers that repeatedly produce low quality studies may be decertified and prohibited from scanning or reading scans from study participants.

6.1 Principle of QA/QC

The purpose of the quality control/quality assurance program is to ensure that the ultrasound data collected for this study are of consistently high quality and are collected according to the study protocol. The foundation of the quality control/quality assurance program rests on a well-defined protocol, standardization of equipment and procedures, and uniform training and certification of sonographers and B-mode image readers. Training and certification procedures are detailed in Section 1 of this document, and the standardized ultrasound protocol is detailed in Section 4. Additional components of the quality assurance program include:

- Monitoring the performance of the Ultrasound System selected for use in this study
- Monitoring sonographer performance to assure consistent acquisition of ultrasound B-mode images
- Monitoring reader performance and equipment to assure consistent measurement of arterial dimensions from the B-mode images
- Preparation of an ultrasound database

6.2 Monitoring Sonographer Performance

The Ultrasound Reading Center will employ a variety of qualitative and quantitative approaches to monitoring sonographer performance, including monthly performance reviews, site visits, comparison of within-sonographer repeat scans, and evaluation of study data.

6.3 Performance Reviews

An annually 5 scans per sonographer be repeated and reviewed for quality assurance of sonographer performance. If the sonographer does not perform a repeat ultrasound study on all participant at its respective site, she/her will be requested to perform them on volunteers. These scans will be sent to the Ultrasound Reading Center using a standard log-in sheet form. This will allow for a review of an overall sonographer performance, which will be grades as:

A) **Satisfactory** - the sonographer has followed protocol and used available tools to maximize image quality.

B) **Marginal** - the sonographer has followed the protocol or has only minor deviations that have minimal impact on the availability and/or quality of arterial measurements.

C) **Unsatisfactory** - the sonographer has deviated from the protocol in a manner likely to affect the availability and/or quality of arterial measurements.

Results and analysis from these performance reviews will be provided to sonographers on a regular basis. Sonographer receiving an overall grade of "C" will automatically be scheduled for retraining with the Ultrasound Reading Center, which will review the scanning protocol and issues with adherence with the protocol with sonographer. After that session (and a conference call) another 3 repeat scans will be assigned for additional review. These scans will require to be scored B or A. If not, the same procedure with direct review with sonographer will be performed. Sonographer will not perform regular research scans until this performance improves to at least score B. If no improvement is achieved on repetitive retraining, the sonographer may be decertified.

1. Unacceptable: only one or no IMT interfaces
2. Poor: only three IMT interfaces
3. Satisfactory: at list four IMT interfaces
4. Excellent: all six clearly visualized IMT

6.4 Monitoring Reader Performance at URC

The Ultrasound Reading Center will use a standard reading station throughout this study.

The Ultrasound Reading Center will employ regular performance reviews, inter- and intra- reader repeat readings, and evaluation of study data as necessary to ensure consistent collection of arterial measurements between.

6.5 Reliability of Reading Procedures at URC

At the URC repeated scans are read twice to allow quantification of CIMT reader sources of variation. For ongoing quality assurance, five percent of the scans will be selected for intra- and inter-reader repeat readings.

6.6 Protection of Study Subjects

The research protocol employed in this study is intended to allow precise estimation of total subclinical atherosclerosis burden (CIMT, plaque) at specific arterial sites, which differs substantially from carotid ultrasound scanning performed for clinical purpose. As a result, the Ultrasound Reading Center is not able to adequately assess the clinical significance of advanced atherosclerotic disease in individual patients and these results cannot be used in clinical practice.

- # Contact information:

Digna Cabral, RVT, CCRP

University of Miami
Department of Neurology
Ultrasound Reading Center
1120 N.W. 14th Street Suite 1362
Miami, FL 33136
Email: dcabral@med.miami.edu
Phone: 305-243-9283
Fax: 305-243-7081

Tatjana Rundek, MD, PhD, FANA

Scientific Director Evelyn F. McKnight Brain Institute
Professor of Neurology
Evelyn F. McKnight Endowed Chair for Learning and Memory in Aging
Director, Clinical Translational Research Division
Email: trundek@med.miami.edu
University of Miami, Miller School of Medicine
1120 NW 14th Street, Suite 1348
Miami, FL 33136
Phone: 305-243-7847

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