Which Tools Are in Your Cardiac Workshop? Carotid Ultrasound, Endothelial Function, and Magnetic Resonance Imaging

Jacques D. Barth, MD, PhD

There are several techniques for assessing arterial health, including carotid ultrasound, endothelial function, and magnetic resonance imaging. Each has pros and cons, but which technique is best? Quantitative intima medial thickness (QIMT) is safe, validated, portable, has a reference database, is inexpensive, and can be used in multicenter studies. Magnetic resonance imaging may be useful clinically, but it is still considered experimental and remains a costly procedure. Flow-mediated dilation (FMD) is a good surrogate measure, reflecting initial risk, indicating very early disease, and demonstrating rapid response to change. All the imaging methods require standardization of tools, operator training, specification of populations studied, and other considerations to be of use in the clinical setting.

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Most health professionals agree on how atherosclerosis develops. A normal, healthy endothelium is exposed to a variety of damaging factors. Eventually, the healthy tissue is injured and endothelial dysfunction results. If the damage persists, raised lesions in the vessel wall and atherosclerotic plaque occur. Plaque vulnerability and likelihood of rupture ensue.[1,2] Familiarity with this process is vital in determination of the use of surrogate markers in clinical trials, as different markers are more appropriate at different stages of cardiovascular disease (Figure 1).

A surrogate endpoint is a biomarker intended to substitute for a clinical endpoint in a clinical trial. However, surrogate markers have some inherent limitations. For example, response may not translate into clinical benefit or survival benefit and may not be intervention dependent, but waiting for the clinical endpoint determination in the preclinical phase can take a decade.[3] Development of surrogate markers allows us to establish prevention measurements during this time frame, hopefully providing us a safe, noninvasive, and reproducible method to assess the progression of this disease. So our main questions are, Can imaging provide a useful surrogate endpoint to identify high-risk subjects? and What imaging technique is best?

There are currently 3 predominant noninvasive imaging methods: quantitative intima media thickness (QIMT), flow-mediated dilation (FMD), and magnetic resonance imaging (MRI). What are the advantages of these various imaging methods?

QIMT is suitable for all stages of the disease and can be used to diagnose and track the disease. The carotid intima media thickness (IMT) has predictive value for likelihood of cerebrovascular and cardiovascular events in men and women.[4] It has been used as the sole surrogate endpoint in the additional approval process of several compounds. In addition, we did a comparison between a QIMT computer software program and caliper IMT. We found that according to standardized measures, the computer program was 4 times as accurate as the caliper IMT (Figure 2). Repeated caliper measurements were, in fact, within the normal variation of the technique, resulting in a significantly less accurate method to track IMT than the ARTIS (arterial imaging system; Prevention Concepts, Inc., West Los Angeles, CA), a computerized edge contour measurement technique.

QIMT is a safe, standardized and validated method that uses ultrasound images. With QIMT, quantitative measurements are possible. It is portable, has a large reference database, and is relatively inexpensive. QIMT has a proven track record and improves patients' adherence to a prescribed regimen. Data show that QIMT correlates with cardiac and cerebrovascular outcome (cardiac is even more accurate than stroke), indicated risk (absolute and relative) of cardiovascular risk factors, as well as the change of risk during management (Figure 3).[5–7] It can be used in all populations throughout the world, including children. QIMT looks at atherosclerosis directly, as with noninvasive intravascular ultrasound sonography (Table 1).

What is the predictive value of QIMT? It varies among different cultural populations.[8] Smoking, blood pressure, and low socioeconomic status are all correlated with carotid IMT.[9–11] Postprandial blood sugar correlates with IMT among nondiabetics, as does duration of disease in diabetics.[12,13] IMT also predicts plaque formation in men and women, independently of baseline plaque.[14] The data derived from the digital analyses can be used in the established database to predict clinical course with or without intervention. In our laboratory, inter- and intrareader

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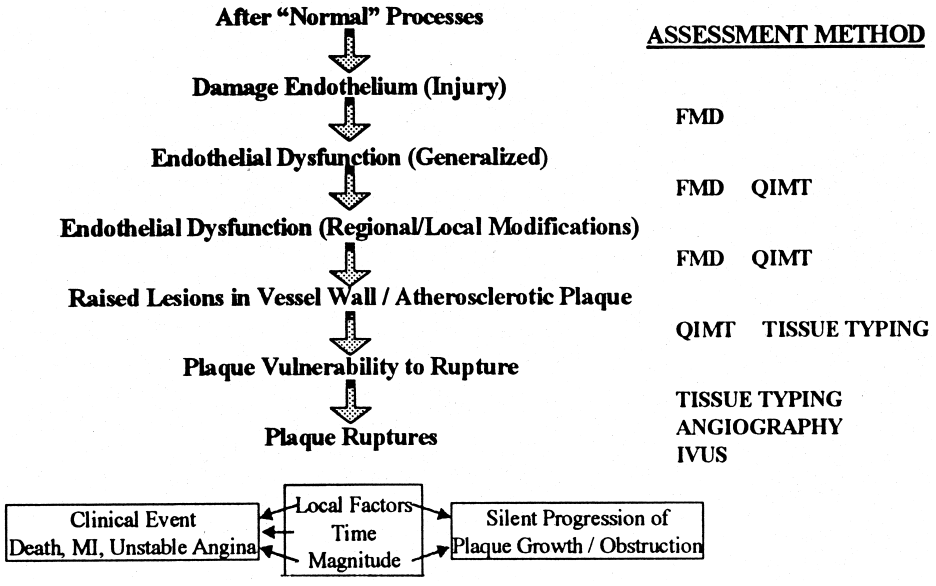

FIGURE 1. Sequence of events leading to adverse outcomes in coronary artery disease if cardiovascular risk factors persist. FMD = flow-mediated dilation; IVUS = intravascular ultrasound sonography; MI = myocardial infarction; QIMT = quantitative intima media thickening.

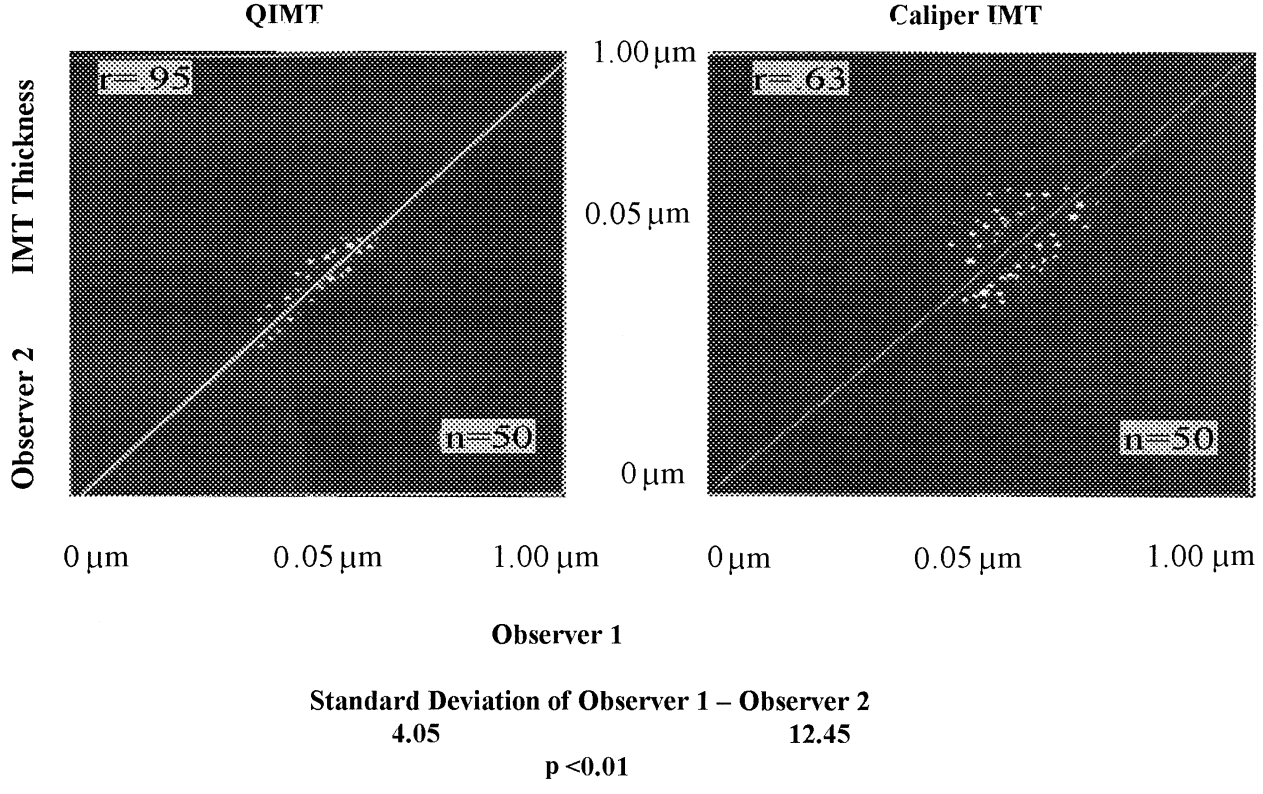

FIGURE 2. Quantitative intima media thickening (QIMT) versus caliper intima media thickening (IMT). Comparison of absolute laser-measured carotid intima media thicknesses by the available user-pointed caliper method with 2 operators and the same measurement by the same experienced 2 operators using the quantitative assessed intima media thickness (ARTIS; Prevention Concepts, Inc., West Los Angeles, CA) procedure.

class correlations were 0.97 in previous studies, and the coefficients of variation within readers were 3.0% and between readers were 3.1%, both of which are considered acceptable.

FMD is a good surrogate measure and reflects initial risk. It indicates very early disease, shows rapid response to change, is ambulatory, and may indicate more of a pathophysiologic response. However, out-

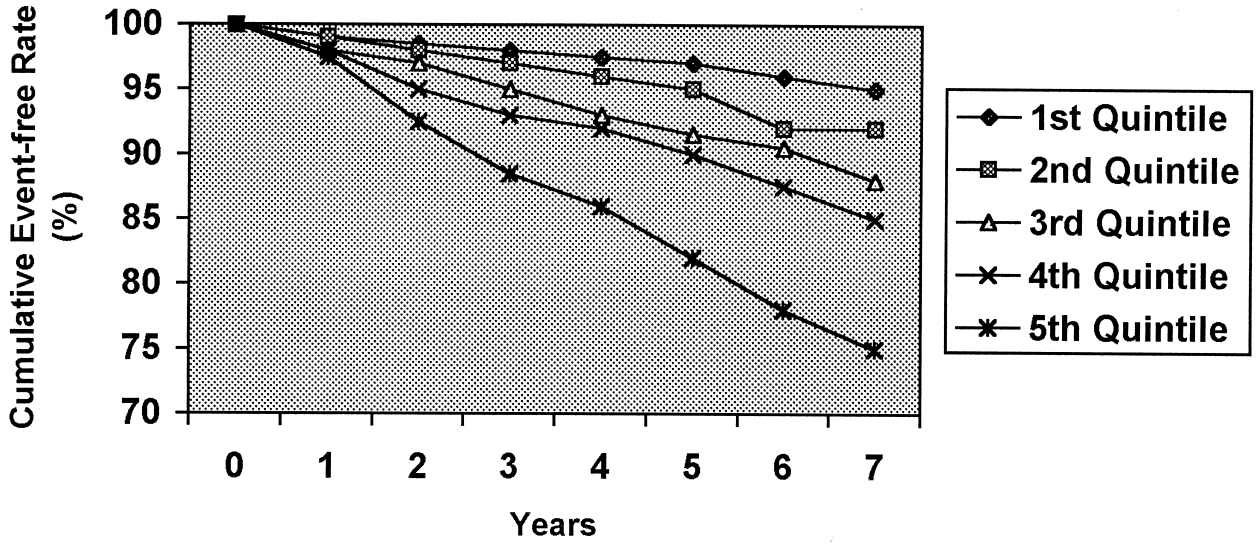

FIGURE 3. Unadjusted cumulative event-free rates for the combined endpoint of myocardial infarction or stroke, according to quintile of combined intima-medial thickness. (Adapted from *N Engl J Med.*[7])

TABLE 1 Quantitative Intima Medical Thickness (QIMT): Advantages and Disadvantages

QIMT Advantages	QIMT Disadvantages
• Safe • Validated, standardized • Quantitative, portable • Proven track record in single-center and multicenter studies • Improves patient adherence • Correlated with CV risk/clinical events (CAD/stroke) • No radiation • All populations (including children) • Reference database • Inexpensive	• "Too simple" • "Access" to standardized laboratory necessary • Certification • Specialized training • QA/QC (internal and external)

CAD = coronary artery disease; QA/QC = quality assurance/quality control.

come studies are lacking. Among its disadvantages are that there is no standard, the procedure is very operator sensitive, and there is no gradual change—there is either dysfunction or no dysfunction.

MRI has high sensitivity and specificity for ex-vivo plaque characterization. MRI in a clinical setting may be feasible in the near future, but for now it seems to be too experimental to consider as an endpoint.[15,16] In addition, the costs of the procedure are too prohibitive to be used as a routine clinical procedure.

Plaque tissue characterization is another evaluation tool. Calcified plaques are not correlated with stroke. Soft plaques have an increased likelihood to rupture and cause stroke.[17,18] Identifying these plaques early may help to predict events (Table 2).

Atherosclerosis is prevalent in all countries, and clinical trials can be conducted globally for this type of disease. Therefore, availability of various tests must be considered, because many countries will not have facilities similar to those in Western countries, thereby limiting the ability to translate findings consistently from one country to another.[19,20]

Another issue that should be considered when planning clinical trials is lack of patient adherence to healthcare provider recommendations. Patients tend to shift responsibility for disease management to their doctor, but doctors need to encourage patients to take responsibility for their own health. We found that patients' adherence to recommendations on diet, exercise, and smoking cessation can be increased if a personalized picture of the artery is provided.[21] Among 210 participants after 12 months, the number of subjects with hypertension decreased from 40 to 14. Hypercholesterolemia was evident in 55 subjects at baseline, and only 10 at the 12th month. The number of subjects who smoked decreased from 22 to 13. There was also a significant decrease in IMT among those given the image of their artery. The conclusion of this study was that those subjects carrying an image of their carotid artery complied significantly better than those without a picture. Picture carrying seemed to be more effective in persistence of behavior modification (Table 3).

We also did a study in young adults of various

TABLE 2 Plaque Tissue Characterization

Risk	Johnson*	Gray-Weale†	Reilly‡	ECPS§
High	Soft	Echolucent	Mixed	Echolucent
Intermediate	Dense	<25% lucent	Intermediate	Thin cap
Low	Calcified	Echorich	Calcified	Echorich

Reprinted from *Arteriosclerosis*.[18]
*See Johnson JM, et al. *Arch Surg*. 1985;120:1010.
†See Gray-Weale AC, et al. *J Cardiovasc Surg*. 1988;29:676.
‡See Reilly LM, et al. *Arch Surg*. 1983;146:188.
§See ECPS (European Carotid Plaque Study Group). *Eur J Vasc Surg*. 1995;10:23.

TABLE 3 Image Compliance Study

	Baseline		12 months	
Personal image	Y	N	Y	N
Hypertension (n)	40	44	14†	21
Hypercholesterolemia (n)	55	52	10†	18
No exercise program (n)	58	58	11†	38*
Smoking (n)	22	20	13†	19
Weight loss (kg)			9 ± 5†	2 ± 4
QIMT values (mm)	0.795	0.783	0.777†	0.790*
±SD (values as compared with baseline)	± 0.069	± 0.072	± 0.062	± 0.067

N = 210 participants.
QIMT = quantitative intima medial thickness.
*$p < 0.05$.
†$p < 0.01$.
Adapted from AHA Compliance Meeting, Waltham, Mass, 1999.[21]

cultural backgrounds to determine if screening in high school was feasible and what abnormalities must be considered. We found that 10% of these high school students (13–17 years old) were hypertensive, 15% were hypercholesterolemic, and 14% already had an arterial lesion. When given the image of their carotid artery, many lost weight, quit smoking, and improved their diet.[22]

A study by another group compared angiography and FMD in evaluation of coronary artery disease in 74 patients versus 14 asymptomatic controls. This study found that FMD was better at identifying the extent of coronary artery disease than angiography or cholesterol level.[23] Another study on FMD compared 34 men with clinical signs of coronary disease, and 33 matched controls without disease. The researchers found a significant negative correlation between FMD and QIMT. They concluded that FMD dysfunction may be a precursor to atherosclerosis.[24]

If we are going to use surrogate imaging endpoints, we need to have a standardized protocol (see Appendix). Training and certification should be standardized, as the technician is the most important link to good imaging. There should be 1 core imaging laboratory not involved in image acquisition, and an image tracking and data trail is vital. Multicenter capability is important, as is quality control and quality assurance in external supervision. Also, a local and a master phantom should always be used.

Drift and randomness are important in evaluating if a site is doing a good imaging job. There are several rules to determine if a technician needs retraining: (1) the M5 rule—if 5 consecutive points are on the same side of the nominal; (2) the 1SD4 rule—if 4 consecutive points are 1 standard deviation above or below nominal; and (3) the 4SD rule—if 2 consecutive points are 4 standard deviations apart.

In summary, all the imaging methods require standardization of tools, operator training, specification of populations studied, and strict quality control to be useful in the clinical setting (Table 4). QIMT/FMD might be added to several ongoing studies to evaluate the best marker for each stage of cardiovascular disease. Initially, FMD of the endothelial function is especially suitable if no obstructive disease is present to focus on a physiologic state. QIMT is the best method if there is endothelial dysfunction and suspected thickening of the far wall of the common carotid when a cardiovascular risk is present. It is especially suited for multicenter, multisite studies. Tissue characterization should be performed if there is plaque formation. QIMT is a useful surrogate for the development of antiatherosclerotic drug therapy.

DISCUSSION

Michael H. Davidson, MD (Chicago, Illinois): Has a change in the surrogate (IMT) been correlated with a reduction in coronary events in a large-term trial?
Jacques D. Barth, MD, PhD (Los Angeles, California): Yes, in the Rotterdam trial (an observational study of 7,983 subjects)[4] they showed that a change,

TABLE 4 Features and Benefits

- QIMT/FMD: (PCI Standard,* ARTIS*)
- Single-center and multicenter application
- Multiple ultrasound equipment
- Cross-calibration and phantom: local/master
- Standardized, local, and centralized training
- Certification, QA/QC external supervisor
- Multisystem CORE laboratories in North America and Europe

CORE = cardiac or respiratory emergency; FMD = flow-mediated dilation; QA/QC = quality assurance/quality control; QIMT = quantitative intima medial thickness.

*Manufactured by Prevention Concepts, Inc., West Los Angeles, CA

for instance, in hypertension also changed the progression rate of IMT and correlated well with the predictability of subsequent coronary complication. If you treated the hypertension or the hypercholesterolemia, for example, the predictive value changed accordingly.

Paolo Raggi, MD (New Orleans, Louisiana): Some of the studies you presented deal with symptomatic patients. Wouldn't that imply preselection bias?

Dr. Barth: If you look at the Rotterdam trial that involved 7,983 patients with a 9-year follow up, I don't think that is preselection bias. There was a whole section of the city that was taking all-comers. This is one of the largest studies. We have done different studies in patients who came into the clinic and wanted to have IMT checked. We currently have an extensive database of >25,000 people, the majority of whom are asymptomatic, and from a variety of ethnic groups. So I don't think there is a selection bias.

Don Black, MD (Ann Arbor, Michigan): For the different modalities you discussed, magnetic resonance imaging, FMD, and QIMT, can you name 1 study that might help determine if these are good predictors?

Dr. Barth: The 9-year follow-up published by Hodis is one of the few studies that combined IMT measurement and coronary angiography. What is interesting, IMT was measured every 6 months and there was never a flip-flop phenomenon. Once there was progression, or stabilization was found in the disease, the same was true for QIMT.

Dr. Black: What about QIMT, FMD, and magnetic resonance imaging to events?

Dr. Barth: For FMD, I'm not certain that there is a study ongoing. One of the biggest problems with FMD is that everyone is using a different technique, and it is very operator dependent. For IMT, the Rotterdam Study is a very powerful study with 7,983 people being observed for 9 years. In the United States, our own extensive database of over 25,000 people with many years of follow-up is quite unique.

David Herrington, MD (Winston–Salem, North Carolina): I'll just respond quickly to the previous question. There are some studies underway to provide the data you mention. All participants in the Cardiovascular Health Study (CHS) cohort had FMD measurements made and now are being observed prospectively for coronary events. FMD was associated with presence of disease cross-sectionally and all patients are being observed prospectively to determine if it is predictive of future events. There is also the Multiethnic Studies of Atherosclerosis (MESA) study, which is the newest National Heart, Lung, and Blood Institute (NHLBI)-sponsored large cohort study in which FMD, carotid IMT, and several other measures of vascular compliance are all going to be measured cross-sectionally in this cohort of 6,500 people and then observed prospectively for 10 years. So, although we don't have answers yet, there are some studies that are currently in process that are going to provide some data. The other caveat is that it is one thing to say something is predictive of future events, which is not the same thing as saying that changing that outcome translates to a change in risk events. That is a distinction that has to be considered with all these tools. The preliminary results will be available in 2008.

1. Virmani R, Kolodgie FD, Burke AP, Farb A, Schwartz SM. Lessons from sudden coronary death: a comprehensive morphological classification scheme for atherosclerotic lesions. *Arterioscler Thromb Vasc Biol* 2000;20:1262–1275.

2. Grundy SM. Primary prevention of coronary heart disease: integrating risk assessment with intervention. *Circulation* 1999;100:988–998.

3. Ogren M, Hedblad B, Isacsson SO, Janzon L, Jungquist G, Lindell SE. Ten year cerebrovascular morbidity and mortality in 68 year old men with asymptomatic carotid stenosis. *BMJ* 1995;310:1294–1298.

4. Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE. Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. *Circulation* 1997;96:1432–1437.

5. Blankenhorn DH, Selzer RH, Crawford DW, Barth JD, Liu CR, Liu CH, Mack WJ, Alaupovic P. Beneficial effects of colestipol-niacin therapy on the common carotid artery. Two- and four-year reduction of intima-media thickness measured by ultrasound. *Circulation* 1993;88:20–28.

6. Hodis HN, Mack WJ, LaBree L, Selzer RH, Liu CR, Liu CH, Azen SP. The role of carotid arterial intima-media thickness in predicting clinical coronary events. *Ann Intern Med* 1998;128:262–269.

7. O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK Jr. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. *N Engl J Med* 1999;340:14–22.

8. Wei M, Gonzalez C, Haffner SM, O'Leary DH, Stern MP. Ultrasonographically assessed maximum carotid artery wall thickness in Mexico City residents and Mexican Americans living in San Antonio, Texas. *Arterioscler Thromb Vasc Biol* 1996;16:1388–1392.

9. Lakka TA, Salonen R, Kaplan GA, Salonen JT. Blood pressure and the progression of carotid atherosclerosis in middle-aged men. *Hypertension* 1999; 34:51–56.

10. Koopman JS, Lynch JW. Individual causal models and population system models in epidemiology. *Am J Public Health* 1999;89:1170–1174.

11. Zanchetti A. Carotid artery wall alterations as intermediate end points. *Clin Exp Hypertens* 1999;21:595–607.

12. Hanefeld M, Koehler C, Schaper F, Fuecker K, Henkel E, Temelkova-Kurktschiev T. Postprandial plasma glucose is an independent risk factor for increased carotid intima-media thickness in non-diabetic individuals. *Atherosclerosis* 1999;144:229–235.

13. Wagenknecht LE, D'Agostino R Jr, Savage PH, O'Leary DH, Saad MF, Haffner SM. Duration of diabetes and carotid wall thickness: the Insulin Resistance Atherosclerosis Study (IRAS). *Stroke* 1997;28:999–1005.

14. Zureik M, Ducimetiere P, Touboul PJ, Courbon D, Bonithon-Kopp C, Berr C, Magne C. Common carotid intima-media thickness predicts occurrence of carotid atherosclerotic plaques: longitudinal results from the Aging Vascular Study (EVA). *Arterioscler Thromb Vasc Biol* 2000;20:1622–1629.

15. Shinnar M, Fallon JT, Wehrli S, Levin M, Dalmacy D, Fayad ZA, Badimon JJ, Harrington M, Harrington E, Fuster V. The diagnostic accuracy of ex vivo MRI for human atherosclerotic plaque characterization. *Arterioscler Thromb Vasc Biol* 1999;19:2756–2761.

16. Hoeg JM. Evaluating coronary heart disease risk: tiles in the mosaic. *JAMA* 1997;277:1387–1390.

17. Geroulakos G, Hobson RW, Nicolaides A. Ultrasonographic carotid plaque morphology in predicting stoke risk. *Br J Surg* 1996;83:582–587.

18. Gronholdt ML. Ultrasound and lipoproteins as predictors of lipid-rich, rupture-prone plaques in the carotid artery. *Arterioscler Thromb Vasc Biol* 1999;19:2–13.

19. Barth JD, Zhang L, Zonjee M. Smoking harms the vasculature in Asians more [abstract]. First American Heart Association Conference on Thrombosis, Atherosclerosis and Vascular Biology; May 2000; Denver: Interlocken.

20. Woo KS, Chook P, Raitakari OT, McQuillan B, Feng JZ, Celermajer DS. Westernization of Chinese adults and increased subclinical atherosclerosis. *Arterioscler Thromb Vasc Biol* 1999;19:2487–2493.
21. Barth JD, Zhang L, Zonjee M. A picture tells a thousand words: a personalized picture enhances a patient's compliance [poster]. AHA meeting on Patient Compliance; April 29–30, 1999; Waltham, Massachusetts.
22. Sanchez A, Barth JD, Zhang L, Zonjee M. Cardiovascular risk factors and disease in young adults. *Atherosclerosis* 2000;152:260–263.
23. Neunteufl T, Katzenschlager R, Hassan A, Klaar U, Schwarzacher S, Glogar D, Bauer P, Weidinger F. Systemic endothelial dysfunction is related to the extent and severity of coronary artery disease. *Atherosclerosis* 1997;129:111–118.
24. Hashimoto M, Eto M, Akishita M, Kozaki K, Ako J, Iijima K, Kim S, Toba K, Yoshizumi M, Ouchi Y. Correlation between flow-mediated vasodilation of the brachial artery and intima-media thickness in the carotid artery in men. *Arterioscler Thromb Vasc Biol* 1999;19:2795–2800.

APPENDIX: STANDARD OPERATING PROCEDURES

Prevention Concepts, Inc. standardized acquisition of common carotid artery: Ultrasonography of both carotid arteries will be performed with a linear array 7.5–10 MHz probe using a duplex B-mode scanner. The ultrasound images are simultaneously recorded on half-inch tape with an S-VHS videotape recorder. The goal is to capture the longitudinal ultrasound image of the distal common carotid artery, including the adventitia, media, and the intima, respectively. The blood containing lumen will appear as black on the image.

The thickness of the intima of the large arteries gradually increases with age. Diffuse intima thickening may be considered a normal part of aging. In some subjects, however, intima thickness increases more rapidly than in others. Hemodynamic factors are one of the major determinants of intima thickening. Thickening of the intima may be a response of the vessel wall to a change in flow (usually a reduction in flow), wall tension (an increase in tension), and shear stress in an attempt to restore normal flow conditions. In particular areas, such as arterial bifurcation (carotid), it is simply a consequence of geography of the artery: large differences in shear stress are present over a small segment of the arterial wall, and adaptive intima thickening occurs.

These sites often coincide with the locations of early development of atherosclerotic lesions and appear to develop whether or not high levels of atherogenic lipoproteins are present. This indicates that atherosclerotic lesions are more likely to develop at sites superimposed on the adaptive process. Turbulence in these areas is more pronounced and clearance of blood particles in these areas appears to be delayed. This results in a longer exposure of the vessel wall to possible atherogenic factors in the blood, which may accelerate the development of early atherosclerosis. Additionally, other factors such as lipid accumulation and vascular injury with thrombus formation have been found to play a major role in the development and progression of arterial atherosclerosis.

Cross-sectional views (slices) of the carotid artery are obtained to image intrusive lesions. Longitudinal views of the common carotid artery, carotid bulb, and internal carotid artery are obtained to image the FAR wall of the common carotid artery and any lesions detected with the cross-sectional view.

Patient positioning:

- To acquire the image, the subject is in supine position.
- The head is turned approximately 45° in the opposite direction of the ultrasonographer (operator). First side of interest is always the **right common carotid artery; place the head accordingly with the right side accessible**. Remove necklaces and other objects that might interfere with the procedure.
- The head and neck should be in line. The special oblique 45°-angle pillow should always be used to standardize the position of the head of all patients. A straight and comfortable position of the head and neck relaxes the neck muscles, which facilitates image acquisition. *Patient comfort speeds exam*. Place a pillow under the knees to reduce low-back discomfort. In case other supporting pillows are needed (e.g., under shoulders etc.), **document exactly what pillows have been used and where they were placed. If no oblique 45°-angle pillow was used, the operator must document this on the label of the subject's videotape and on a Data Action Sheet. If no pillow is used for the baseline scans, no pillow should be used for any follow-up scans to insure consistent patient positioning.**
- The subject should be lying with head at the very top of the scanning table (close to operator). The operator may prefer to sit at the top of the table above the subject's head with the machine within reach. In this position, the operator's forearm can rest on the bed and the hand holding the probe can be positioned in a stable manner. This position is usually more comfortable for the operator, and also stabilizes the probe and therefore the image. Extend the neck slightly by lifting the chin. Check that the tension of the neck muscles (sternocleidomastoid) is low.
- The location of the bifurcation may vary between subjects and can sometimes be located very high, just under the cheek. The position of the internal carotid artery and external carotid artery may vary. In approximately 50% of the cases, the internal carotid artery has a posterolateral origination. The angle of the bifurcation dividing into the internal carotid artery and external carotid artery may vary and may cause difficulties in imaging both arteries. The bulb is usually located in the proximal internal carotid artery, but can vary from patient to patient to include the distal common carotid artery and/or the proximal external carotid artery.

Scanning procedures:

- Turn on the ultrasound machine, video recorder, and the video printer.
- Wait before using the ultrasound machine for at least 60 seconds until it is warmed up. Use this time to obtain a new S-VHS tape and **label the tape with the site name, study ID, patient ID, patient initials, birth date, and the date of the scan.** Record only 1 subject on 1 tape. The follow-up visits will be recorded sequentially onto the same tape. Each subject has his or her own videotape! **Check to be sure the video recorder is recording at STANDARD speed.**
- Check the screen monitor contrast and brightness. It should be fixed in the mid-position**. Once set, do not readjust the contrast and brightness.**
- **NEVER use the ZOOM function.**
- Select the dedicated program for this carotid ultrasound procedure (preprogrammed settings at specific carotid body mark). Check to be sure that data currently on the screen are correct for this protocol.
- Select B-Mode.
- Check DEPTH GAIN CONTROLS. This adjusts the intensity of the ultrasound echo in the shallow (NEAR) and deep (FAR) areas. All depth gain controls should be fixed at mid-range position. **Do not set the GAIN knob at an excessively high level, because the image echo might saturate and disable observation. The vessel lumen should be black and no noise visible in the image. Noise in the vessel lumen might interfere with the analysis.**
- Check the DEPTH setting. The carotid arteries are located approximately 2–3 cm below the skin. The DEPTH should preferably be fixed **at 4 cm** but some patients will require increased depth.
- Set FOCAL ZONE at or just below area of interest.
- Check ZOOM mode. Set to normal.
- Properly position subject in the supine position on the examination table.
- **PRINT OUT THE MACHINE SETTINGS FOR REFERENCE ON FOLLOW-UP SCANS.**
- Select NEW PATIENT from keyboard and enter the patient biography information using the following abbreviations: (1) PATIENT ID (PID), (2) PATIENT INIT (PIN), (3) BIRTH DATE (BD), (4) STUDY ID (SID), (5) VISIT number (Vnumber), (6) SITE/VIEW (SV), (7) OPERATOR ID (OID).
- Place **sufficient** ultrasound gel in the area of scanning. **Insufficient gel** will influence the transmission of ultrasound waves and the image will be poor!
- **Start the video recorder.** The video recorder should be set to record at **STANDARD SPEED.** The total scanning procedure (right + left) should not take >20 minutes of recording time.

Cross-sectional view: Place the probe cross-sectional just above the clavicle and move it laterally until the image of the common carotid artery is positioned below the jugular vein and both vessels are in the **center** of the image. Move the probe until **the jugular vein is on top of the carotid artery**. Sometimes you will have to position the probe more lateral and posterior to get both vessels on top of each other. Sometimes the jugular vein collapses and is hard to detect. Ask the subject to hold their breath and bear down (as if they were having a bowel movement). This will inflate the jugular vein and it will give a clear view of the location of both vessels. Scan slowly toward the head to image the entire carotid system. Image the bifurcation, the internal carotid artery and the external carotid artery up to 1 cm beyond the bifurcation.

- Any lesions visible should be marked using the annotation panel. Freeze the image and enter an asterisk symbol 1 cm above the lesion in the black area (blood). This will identify any observed lesions by the operator on site.
- Lesions in the bifurcation area are of special interest; therefore, the operator should scan this area carefully.
- Scan toward the clavicle again and visualize the mid- and proximal common carotid artery such that the double line pattern is clearly visible in the FAR wall.
- Scan toward the bulb again and **just proximal of the bulb, slowly turn the probe counterclockwise to scan longitudinally. During this turning procedure, focus on the double lining in the FAR wall of the distal common carotid artery and keep this clear in your image. This procedure will open up the distal common carotid artery and the jugular vein will still be on top and parallel**.

Longitudinal view:

- Enter SITE as **R** (right) or **L** (left).
- Position the probe laterally with a 90° angle and tilt with very small movements in the anterior or posterior direction.
- In the longitudinal view, the common carotid artery should be horizontal and the jugular vein should be positioned exactly parallel above the common carotid artery. This *horizontal and parallel positioning* of the common carotid artery and jugular vein ensures that the probe is perpendicular to the FAR wall.
- Make sure that the left side of your screen correlates with the cranial side of the subject. **The bulb should always be positioned on the left side of the screen and the right side of the screen in the direction of the heart. Please make sure this is done consistently for all scans!**
- Slowly scan toward the head until the bifurcation is clearly visible. The bifurcation is in conjunction of the area of the bulb. **This is the area of interest for the QIMT measurement.**
- Maneuver the probe until 0.5 cm of the proximal part of the bulb is present at the left side of the screen. *This 0.5 cm part of the bulb* should be included in all images focusing on this area. This portion of the bulb is used as an anatomical reference point for the quantification procedure and all follow-up scans.

Measurements acquisition	Baseline 1: Brachial diameter (1,2 minute) & flow (5-30 seconds). Cuff Phase: Brachial diameter & flow at 1,2,3,4,5 minutes (30 each).

- Obtain an **optimum image** of the bulb and distal part of the common carotid artery.
- An optimum image means:
- *A bright continuous double line at the FAR wall of the distal common carotid artery.* Try to reposition the probe until the *longest* continuous double line is present in this part of the common carotid artery.
- *Parallel horizontal common carotid artery and jugular vein.* If the jugular vein is not clearly visible, the subject should hold their breath and bear down (as if having a bowel movement).
- *Probe is positioned perpendicular to vessel walls.* Minimal noise in the image with the lowest gain possible.
- *Record this optimum image for at least 10 seconds.* This 10-second recording will provide several images for the core lab to select from when digitizing images.

A weekly phantom taping is requisite for optimal acquisition. A biannual gold standard phantom take is critical.

A Prevention Concepts, Inc. standardized acquisition of flow-mediated dilation: Ultrasonography of the brachial artery will be performed with a linear array 5–7.5 MHz probe using a duplex (B-mode and Doppler) ultrasound scanner. The ultrasound images are simultaneously recorded on half-inch tape with a S-VHS videotape recorder. The goal is to measure changes in lumen diameter and flow pattern from baseline to 5 minutes after induction of hyperemia. The crucial measurement is the distance between the NEAR and FAR wall echo interfaces of intima lumen and lumen intima, respectively, in the brachial artery.

The brachial artery is much smaller than the carotid artery and often located more superficial and within 1–2 cm deep. The brachial artery is located in the cubital area, lateral of the internal epicondyle of the humerus and medial of the biceps tendon. The first step to locate the artery is palpating the cubital area and marking the spot with the strongest palpations. With only B-mode, it might be difficult to locate the brachial artery. Pulsation of tissue may be the only clue to the location of the brachial artery, which is often located <2 cm deep. Using pulsed wave Doppler will facilitate identification of the brachial artery. Detection with ultrasound is only possible over a short segment when it runs superficial. The blood containing lumen will appear black on the image and pulsate. To measure the *diameter* of the brachial artery, a *horizontal* brachial artery with double lining of both the *NEAR wall and FAR wall* should be clearly visible. Tilting of the transducer is necessary to obtain the brachial artery at a *60° angle* with the Doppler signal needed for *velocity measurements*. Recording optimal images of the brachial artery in B-mode and with Doppler will be used for baseline measurements. Occlusion of the brachial artery for 5 minutes by inflating the blood cuff will cause ischemia. After release of the cuff, continuous recording for 5 minutes with alternate B-mode and Doppler images will be performed.

Patient positioning: To acquire the image, the subject is in sitting position with the *nondominant* arm fully extended on the exam table facing the cubital area.

- Palpate in the antecubital area, the brachial artery medial of the biceps tendon, and lateral of the epicondyl.
- *Mark* the *exact position of the artery* with the dermatologic marker.
- Place the arm in the splint and use the vacuum pump to inflate the splint and stabilize the arm.
- Check if the arm is not squeezed in the splint. Use the straps to stabilize the arm but make sure the area with the mark has clear and sufficient space left for the probe.
- Attach the blood pressure cuff above the splint and position the cuff pump in such a way that it is easy to have access during the scanning procedure.
- ***MEASURE THE BLOOD PRESSURE*** and document the blood pressure on the screen of the ultrasound machine (**BP1**).
- Make sure the patient is sitting comfortably and the arm is positioned comfortably.
- Scan the marked area and locate the brachial artery. Focus on pulsating tissue and image the brachial artery horizontal with a "clear" vessel lumen and clear **double lining** in both the **NEAR and FAR** walls as optimally as possible. The brachial artery is not straight over a very long segment in this area. Try to image a horizontal segment with a clear NEAR wall and FAR wall and clear vessel lumen.
- Pulsed wave Doppler in a clearly defined and visualized vessel will facilitate the appropriate angle cursor placement with the axis of the vessel (i.e., parallel to the vessel walls). If the vessel is not well visualized or is tortuous so that the angle cannot be well defined, the velocity measurement should be questioned. Tilt the probe to get a 60° angle.

1. Position patient in supine position comfortably so that the *nondominant* arm can be studied, and allow him or her to rest undisturbed.
2. Attach 3-lead electrocardiograph. Make sure QRS has a prominent R wave with a voltage >1 cm.
3. Attach blood pressure cuff to nondominant arm for blood pressure measurements.
4. Attach blood pressure cuff to nondominant arm (upside down and as proximal as possible). If patient's arm is too large, use a cuff of appropriate size. The cuff tubing will be pointing toward the patient's shoulder.
5. Labels will include study number, subject's ID number, principal investigator's name, date, and visit. Use a 120-minute SVHS tape to record studies.
6. Patient's arm will be outstretched on board, and secured palm up, 45–90° from their body, depending on patient's comfort and quality of images.
7. Type patient information at the screen: study number, subject's ID number, subject's initials, center number, date of birth, arm (right/left), and visit number.

Prebaseline imaging: It is crucial to optimize the 2-dimensional images and Doppler signals *before* the actual study starts.

1. Locate brachial artery pulsation approximately 2–5 cm above interepicondylar line (antecubital fossa) skin fold. Use whichever landmark is more readily identifiable. Place transducer at the point of maximal pulsation. Document the distance (cm) from the landmark to the probe. You will be scanning the patient in the exact same place on both visits. Optimize depth (preferably 3 cm) and gain for best lumen image quality.
2. Color flow will be used to identify vessel. Turn the color on and image the brachial artery in long axis.
3. Optimize 2-dimensional image so that the artery is positioned *horizontally* and intimal lines are seen in both the anterior and posterior edges of the vessel. This ensures that the probe is perpendicular to the main axis of the vessel, avoiding tangential cuts. Avoid noise in the lumen.
4. Arterial flow velocity will be obtained using pulsed Doppler at a documented 60° angle of incidence corrected to the direction of blood flow. The range gate (1.5 mm) will be positioned in the center of the arterial lumen. Check if position is correct and signal triphasic and maximal.

Baseline recordings number 1:

- Type "BASE-1" at the bottom center of the screen to indicate baseline period.
- Intermittently turn on color to identify the vessel only. During scanning B-mode use color as little as possible.
- The use of color Doppler interferes with the computer measurements of diameter and should be used carefully and sparsely.
- Start recording (exactly at minute interval–00 seconds). DO NOT STOP recording throughout the entire study.
  - A. Image vessel in horizontal position in B-mode continuously for 1 minute.
  - B. At the end of 1 minute, switch to pulsed wave Doppler and place the sample volume in the middle of the vessel and carefully optimize flow recordings.
  - C. Obtain flow readings for at least 5 seconds (up to 30). On the spectral display, baseline should be placed at one quarter of the screen. Doppler filter should be OFF. Set sweep speed at 50 mm/second.

Repeat Steps 15A through 15C for the second minute of baseline number 1 recordings.

Cuff phase:

1. Change text on screen to read "CUFF."
2. Inflate cuff up to 50 mm Hg above systolic blood pressure to occlude the artery **(blood pressure has to reach a minimum of 200 mm Hg)**. Maintain cuff inflation for **5 minutes**.
3. Deflate the cuff completely.
4. Mark "Time = 0 min" on screen. Document exact time (hh:mm:ss [hours: minutes:seconds]) on screen and data sheet.
5. Start with B-mode. Image the artery horizontally for 30 seconds with clear and parallel walls.
6. Switch to pulsed wave Doppler and position sample correctly. Measure the highest peak velocity.
7. Image the vessel for 5 minutes, alternating between B-mode and pulsed Doppler for 30 seconds each. Start with B-mode.