Clinical Application of Noninvasive Vascular Ultrasound in Cardiovascular Risk Stratification: A Report from the American Society of Echocardiography and the Society of Vascular Medicine and Biology

Represented by Mary J. Roman, MD, Tasneem Z. Naqvi, MD, FASE, Julius M. Gardin, MD, FASE, Marie Gerhard-Herman, MD, Michael Jaff, DO, and Emile Mohler, MD

EXECUTIVE SUMMARY

Noninvasive measures of atherosclerosis have emerged as adjuncts to standard cardiovascular disease (CVD) risk factors in an attempt to refine risk stratification and the need for more aggressive preventive strategies. Two such approaches, carotid artery imaging and brachial artery reactivity testing (BART), are ultrasound based. Numerous carotid artery imaging protocols have been used, and methodologic aspects are described in detail in this review. The panel recommends that protocols: (1) use end-diastolic (minimum dimension) images for intimal-medial thickness (IMT) measurements; (2) provide separate categorization of plaque presence and IMT; (3) avoid use of a single upper limit of normal for IMT because the measure varies with age, sex, and race; and (4) incorporate lumen measurement, particularly when serial measurements are performed to account for changes in distending pressure. Protocols may vary in the number of segments wherein IMT is measured, whether near wall is measured in addition to far wall, and whether IMT measurements are derived from B-mode or M-mode images, depending on the application. BART is a technique that requires meticulous attention to patient preparation and methodologic detail. Its application is substantially more challenging than is carotid imaging and remains largely a research technique that is not readily translated into routine clinical practice.

From Weill Medical College of Cornell University, New York, NY (M.J.R.); Cedars Sinai Medical Center, Los Angeles, CA (T.Z.N.); St. John Hospital & Medical Center, Detroit, MI (J.M.G.); Brigham and Women's Hospital, Boston, MA (M.G-H.); Massachusetts General Hospital, Boston, MA (M.J.); University of Pennsylvania Health System, Philadelphia, PA (E.M.).

Reprint requests: The American Society of Echocardiography, 1500 Sunday Dr, Suite 102, Raleigh, NC 27607. (919) 864-7754.

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CAROTID ULTRASOUND IN CARDIOVASCULAR RISK STRATIFICATION

Carotid ultrasonography has traditionally been used to evaluate the presence of obstructive atherosclerosis in the setting of symptomatic cerebrovascular disease or asymptomatic carotid bruit. More recently, carotid ultrasonography has been performed in epidemiologic studies to measure IMT and detect nonobstructive plaque to evaluate the relation of these findings to CVD risk factors and CVD morbidity and mortality. In addition, changes in carotid IMT may be used as a measure of efficacy of pharmacologic intervention. The prognostic significance of these findings and their potential use in refining CVD risk stratification will first be briefly discussed.

Prognostic Use of Carotid Ultrasound

A number of longitudinal studies involving population-based samples in different countries have examined the relation of baseline carotid IMT and/or discrete plaque to subsequent CVD event rates (Table).[1-6] The prognostic studies have varied in methodology: IMT and plaque are not always evaluated separately; risk is variously stratified based on thresholds, quintiles, SD, and/or increments of IMT; and multivariate analyses including standard CVD risk factors have not always been applied to examine the independent or additive use of carotid ultrasound findings. Thus, although these studies demonstrate that carotid IMT and/or plaque predict increased risk, the ability to translate results to other populations or individuals is somewhat limited by differences in methodology and statistical approaches. Those studies that have analyzed IMT and plaque separately show the greatest risk of future myocardial infarction to be conferred by the presence of focal plaque, rather than increased IMT.[2-4] When traditional CVD risk

Table Population-based prospective studies of the prognostic use of carotid ultrasonography

Study	Population	n (% male)	Age, yr	Race	Follow-up, yr	Carotid USG parameters	End point	Results
KIHD[2]	Eastern Finland	1257 (100)	42, 48, 54, 60	C	2	Normal CCA IMT > 1.0 mm Nonstenotic plaque Stenotic plaque	MI	No increased risk with IMT > 1.0 mm Plaque: RR: 4.1 (1.8–9.2)
KIHD[3]	Eastern Finland	2181 (100)	42, 48, 54, 60	C	4	Same as above	MI	IMT > 1.0 mm: RR: 2.1 (1.1–4.1)* Nonstenotic plaque: RR: 3.4 (1.9–5.9)* Stenotic plaque: RR: 6.3 (3.1–12.6)*
Chieti and Pescara[4]	Italy	2000 (56)	30-70	C	6	Same as above	Incidence of MI, angina, CVA, PVD	IMT > 1.0 mm: 5.5% Plaque: 18.4% Stenotic plaque: 42%
ARIC[5]	United States	12,841 (43)	45-64	C, AA	5.2	Mean IMT of 6 sites	MI, CHD death	IMT < 1.0 mm vs IMT ≥ 1.0 mm Women: HRR: 2.62 (1.55-4.46)† Men: HRR: 1.20 (0.81-1.77)†
Rotterdam Study[6]	Holland	1470 (38)	≥55	C	2.7	SD increase in CCA IMT	MI	Women: OR: 1.26 (0.89-1.79)† Men: OR: 1.25 (0.91-1.72)† IMT > 0.908 mm: OR: 1.44 (0.65-3.16)†
CHS[1]	United States	4476 (39)	>65	C, AA	6.2	Quintiles or SD of maximum CCA and/or ICA IMT	MI, stroke	Increasing risk per quintile or SD†

AA, African American; *ARIC*, Atherosclerosis Risk in Communities; *C*, Caucasian; *CCA*, common carotid artery; *CHD*, Coronary heart disease; *CHS*, Cardiovascular Health Study; *CVA*, cerebrovascular accident; *HRR*, hazard rate ratio; *ICA*, internal carotid artery; *IMT*, intimal-medial thickness; *KIHD*, Kuopio Ischemic Heart Disease Risk Factor; *MI*, myocardial infarction; *OR*, odds ratio; *PVD*, peripheral vascular disease; *RR*, relative risk; *USG*, ultrasonography.

*Results of Cox regression with Cardiovascular disease risk factors not reported, but results said to still be significant.

†Adjusted for Cardiovascular disease risk factors.

factors are considered, the association of baseline carotid artery findings with outcome is usually attenuated, but remains significant,[1,3,5,6] particularly in women.[5]

Interestingly, although carotid artery atherosclerosis is a manifestation of cerebrovascular disease, the majority of events predicted are a result of coronary heart disease (CHD), underscoring the systemic nature of atherosclerosis. Autopsy studies have shown reasonable correlations between the severity (not presence) of carotid and coronary atherosclerosis.[7-9] Similarly, clinical studies have related carotid atherosclerosis detected by ultrasonography to obstructive coronary artery disease diagnosed by contrast angiography[10-12] or clinically manifest CHD.[13] However, in view of the limited ability of coronary angiography to detect significant nonobstructive mural atherosclerosis, the association between the presence of coronary and carotid atherosclerosis is certainly even stronger than that suggested by the existing literature. A study comparing the presence of coronary atherosclerosis detected by intravascular ultrasound and carotid atherosclerosis detected by carotid ultrasound would provide more comparable data and, therefore, more precise information about the strength of association of atherosclerosis in both beds.

Examination Technique

Carotid ultrasound can be performed using standard ultrasound machines equipped with high-frequency transducers (usually 5-12 MHz and linear array) and appropriate software. Standard transducers used in adult echocardiography (2.0-3.5 MHz) do not provide adequate near-field resolution for superficial

vascular imaging. Ideally, the system should allow full-screen display of M-mode images if this modality is used in measurement of IMT and lumen diameters. Patient preparation and positioning are similar to that described in the preceding article. In brief, the patient should be supine with slight hyperextension and rotation of the neck in the direction opposite the probe. The common carotid artery (CCA) is identified in the transverse or longitudinal plane and scanned from its origin to bifurcation. The internal carotid artery (ICA) and external carotid artery are identified using standard anatomic and Doppler features (discussed in the preceding article). Scanning in the transverse plane and from multiple angles optimizes detection of nonobstructive plaque.

Measurement Techniques

IMT: CCA versus other segments. The IMT can be measured in the CCA, the bifurcation (bulb), and either of the branch vessels (usually the ICA). Because of its tubular shape, perpendicular location relative to the transducer beam, and virtually universal accessibility, measurement yield and reproducibility of the CCA IMT are higher than for the ICA[14-16] or bulb IMT.[15-17] In the Atherosclerosis Risk in Communities Study involving carotid ultrasound examinations in 13,824 individuals, IMT measurements were obtainable from the CCA in 91.4%, from the bifurcation in 77.3%, and from the ICA in 48.6% of participants.[15] A report from the Rotterdam Study (n = 1881 in the analysis) showed a similar trend in measurement yield: 96% in the CCA, 64% in the bifurcation, and 31% in the ICA.[18]

One consideration in choosing the segment or segments to measure might be differences in the extent to which IMT of a given vessel correlates with prevalent CVD and/or outcome. In the Cardiovascular Health Study, the combination of CCA and ICA IMTs resulted in minimally higher adjusted relative risks for subsequent myocardial infarction or stroke than did CCA or ICA IMT alone (1.36 vs 1.27 and 1.30, respectively, for 1 SD increase).[1] In this study, ICA IMT had marginally higher adjusted relative risk for prediction of incident myocardial infarction (1.34 vs 1.24), whereas CCA IMT was slightly better at predicting stroke (1.28 vs 1.25). These findings mirror earlier results from Cardiovascular Health Study wherein ICA IMT was a stronger independent correlate of prevalent CHD, whereas CCA IMT was a stronger correlate of prior stroke.[19] Similarly, The British Regional Heart Study noted that CCA IMT was a stronger correlate of prevalent stroke than was bifurcation IMT; the latter was not associated with prevalent CHD when the presence of plaque (measured separately from IMT) was considered.[20] In the Insulin Resistance Atherosclerosis Study, the presence of diabetes and fasting glucose were associated with CCA IMT but not ICA IMT.[21]

On balance, there does not appear to be compelling evidence to suggest that combined measurements or measurement of a specific segment is clearly superior. The higher yield and superior reproducibility of measurement of the CCA IMT, and its better suitability for semiautomated measurement, favor its use, particularly in protocols that do not incorporate plaque (usually seen in the bifurcation or ICA) in the IMT measurement. In such circumstances, the predictive value of focal atheroma in these areas is preserved (by the categoric presence of plaque) without sacrificing measurement yield or accuracy (by attempting to measure bifurcation or ICA IMT).

IMT: Far wall versus near wall. The IMT may be measured from the near (closest to the transducer) and/or the far wall. Although measurement reproducibility of the near and far walls has been reported to be comparable,[17] measurement yield of the near wall is lower[16] and accuracy may be less than that of the far wall because of technical considerations. Current technology does not permit reliable separate measurement of the intima and media; hence, the standard measurement is combined IMT, which has been anatomically validated for the far wall.[22,23] Excess gain or blossoming of the highly echogenic near-wall adventitia into the echolucent media, or of the echogenic near-wall intima into the echolucent lumen, will result in systematic undermeasurement or overmeasurement, respectively, if IMT of the near wall is measured. In contrast, incursion of echoes from the far-wall intima into the media will not influence overall IMT measured from the far wall. Thus measurement of the far wall is likely to be more accurate than measurement of the near wall.

IMT: B-Mode versus M-Mode measurement. IMT has most commonly been measured from B-mode images (Figure 1). Alternatively, B-mode–guided M-mode images of the distal CCA may be obtained (Figure 1). In either case, because of the very small dimensions, wall thickness should be measured using computer assistance with electronic calipers or semiautomated edge-detection algorithms.[24,25] Although spatial resolution is comparable with the two techniques, temporal resolution is far superior with M-mode imaging, thereby facilitating standardization of measurements at the time of minimum diameter, when the diastolic distending pressure is known, and estimation of pulsatility or vascular function. Instantaneous changes in pressure and diameter can be assessed from continuous tracing of M-mode images and simultaneous pressure waveforms of the contralateral carotid artery obtained using applanation tonometry.[26]

IMT: Timing of measurement. Regardless of whether IMT is measured from B-mode or M-mode images, cyclic variations in IMT and lumen diameter should be taken into account by electrocardiographic gat-

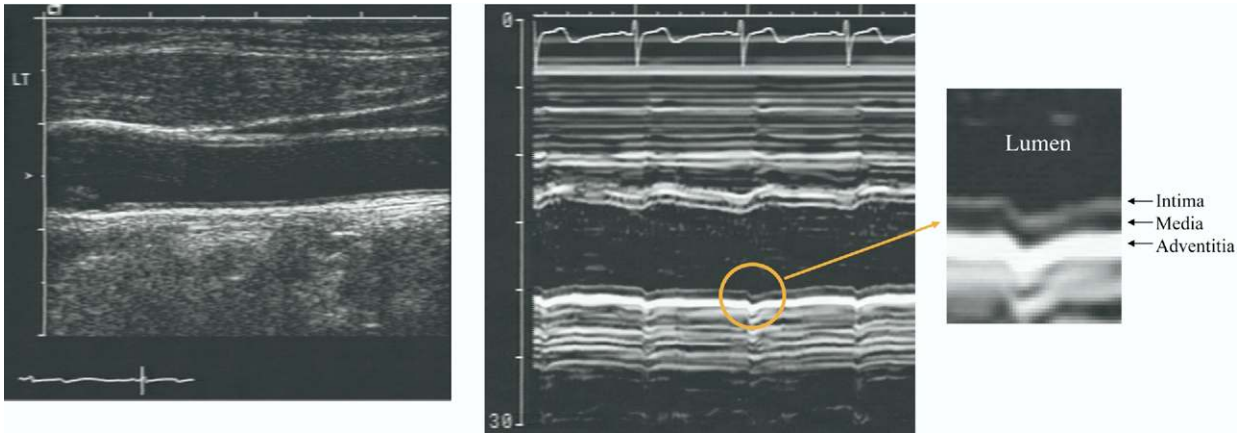

Figure 1 B-mode (*left*) (cephalad to left) and M-mode (*right*) images of distal common carotid artery.

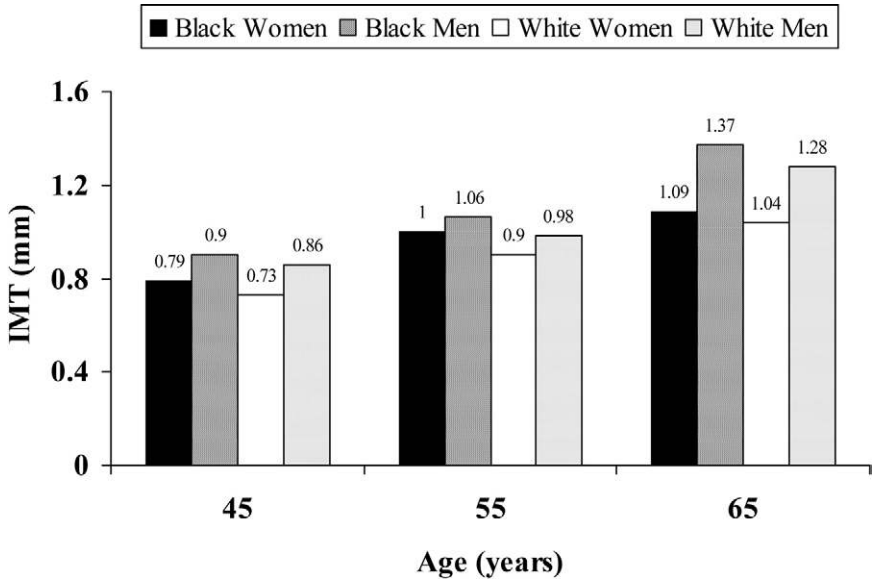

Figure 2 Bar graph of 95th percentiles of common carotid artery intimal-medial thickness (*IMT*) in men and women stratified according to decade of age and race. Adapted.[15]

ing and/or determination of minimal (end-diastolic) and maximal (peak systolic) diameters. With systolic expansion of lumen diameter, obligatory thinning of IMT will occur through conservation of mass (although some degree of longitudinal stretch will occur).[27,28] Systematic timing of measurements is particularly important in serial study and/or intervention trials wherein the magnitude of change in measurements may approximate that seen in cyclic variation in IMT and lumen diameter.

Definition of abnormal IMT. IMT increases with age and, on average, is larger in men than women.[15,20,29,30] In addition, modest racial differences in IMT have been reported. African Americans have higher CCA IMT values than Caucasians[15,30] or non-Hispanic whites[31] who, in turn, have slightly higher wall thicknesses than Hispanics.[31] In the Insulin Resistance Atherosclerosis Study, there were no significant differences in ICA IMT between African Americans, non-Hispanic whites, and Hispanics.[31] Furthermore, systolic blood pressure is an important determinant of IMT, presumably as a result of medial hypertrophy.[15,20,29,32] Thus, a single threshold value for abnormality (eg, 1 mm) may result in systematic underdetection of abnormality in younger individuals and overdetection in older individuals. Ideally, age-, sex-, and race-adjusted thresholds derived from large population-based studies should be used to detect IMT (Figure 2).[15]

The extent to which carotid IMT is a manifestation of early or diffuse atherosclerosis, as opposed to smooth-muscle hypertrophy and/or hyperplasia in-

duced by pressure overload and/or age-related sclerosis, is uncertain. IMT has often been considered a manifestation of atherosclerosis, in view of the relations of IMT to CVD risk factors and to prevalent and incident CVD. Furthermore, in measurement protocols that allow incorporation of plaque thickness into the IMT measurement, IMT is, by definition, a measure of atherosclerosis. However, in protocols in which CCA IMT is measured with separate categorization of plaque, a clear dissociation between increased IMT and discrete carotid atherosclerosis has been demonstrated in relatively young patients with systemic lupus erythematosus,[33,34] suggesting that IMT may not always represent a surrogate measure of atherosclerosis.

Lumen diameter. Internal diameter of the vessel lumen (usually the CCA) can be measured at a single point in time from B-mode images, or throughout the cardiac cycle from M-mode tracings. Determination of minimum and maximum lumen diameters is mandatory for assessment of vascular mechanics.[35] In addition, measurement of lumen diameter and IMT permits calculation of vascular cross-sectional area, a surrogate measure of vascular mass comparable with left ventricular mass.[29] Such calculations may be particularly informative in intervention studies, particularly those using blood pressure-lowering agents. Depending on the mechanism of drug action, comparable degrees of blood pressure lowering may be result in: (1) smooth-muscle relaxation, resulting in a decrease in IMT and increase in lumen diameter with no overall change in vascular mass; (2) a simple decrease in distending pressure resulting in an increase in IMT and decrease in lumen diameter with no overall change vascular mass; or (3) a true regression in vascular mass as a result of decrease in IMT unrelated to changes in lumen diameter (Figure 3). Thus serial measurement of only the IMT may produce misleading results (ie, either falsely positive or falsely negative) for detecting reduction of wall mass.

Nonobstructive plaque. Nonobstructive plaque, which may be defined as the presence of focal thickening at least 50% greater than that of the surrounding vessel wall,[36] is usually readily identifiable, with the best view of its encroachment into the lumen detected from the transverse plane. The most common location of plaque is within the carotid bifurcation, when flow becomes less laminar, followed by the ICA[37]; plaque is much less common in the CCA because of its usually laminar flow profile. Because Doppler velocity does not usually increase until significant (>50%) luminal obstruction develops, nonobstructive plaque cannot be reliably quantified using Doppler techniques. Because of its complex 3-dimensional nature, the size of a single plaque or overall plaque burden is difficult to quantify; thus, the categoric presence of plaque is more

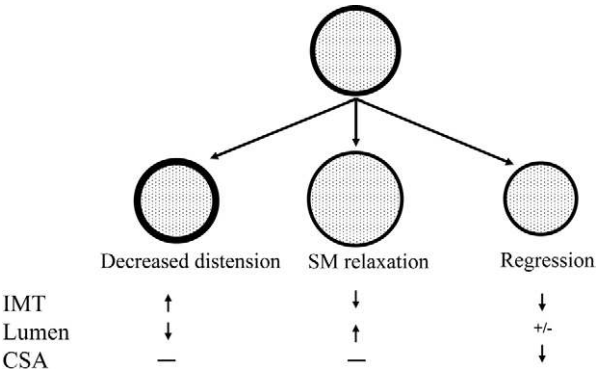

Figure 3 Potential changes in carotid artery anatomy associated with different mechanisms of blood pressure lowering. *CSA*, Cross-sectional area; *IMT*, intimal-medial thickness; *SM*, smooth muscle.

reproducible than measurement of its thickness.[38] Plaque diameter (ie, maximum incursion into the vessel lumen) may be measured, but may not accurately reflect overall plaque burden. Some measurement protocols incorporate plaque diameter into IMT and do not make a distinction between IMT and plaque.[1,19] Other protocols have separated IMT measurement and plaque presence and have used a semiquantitative approach based on the presence or absence of nonobstructive or obstructive plaque.[2-6,39] Alternatively, the number of discrete plaques, or the number of segments of the extracranial carotid arteries containing plaque, may be quantified.[39,40]

Plaque characterization. Reliable characterization of plaque tissue content and of features suggestive of plaque instability (ulceration, thin fibrous cap) is not yet available using standard carotid ultrasound techniques. In general, plaques may be characterized as homogeneous (ie, of uniform echogenicity) or as heterogeneous. Highly echogenic portions of heterogeneous plaques may correspond to areas of calcification, whereas echolucent areas may represent either lipid or hemorrhagic content. The presence of significant calcification is indicated by shadowing, or a signal void beyond the highly echogenic calcium. Quantitative tissue characterization using integrated backscatter analysis to distinguish between predominantly fatty versus fibrous plaque content has been reported.[41] Another potential technique to characterize plaque content and activity is contrast-enhanced ultrasound to detect inflammation. Activated leukocytes attached to the inflamed vessel wall may bind the shells of lipid microbubbles, which are detectable by ultrasound.[42] Recent preliminary reports also suggest that contrast enhancement of the carotid artery lumen–wall interface may improve the ease and accuracy of performing IMT measurements.[43]

Potential Indications for Risk Stratification

The Prevention Conference V sponsored by the American Heart Association addressed the use of carotid ultrasound to identify patients at high risk for primary prevention of CVD. The writing group concluded, "In asymptomatic persons >45 years old, carefully performed carotid ultrasound examination with IMT measurement can add incremental information to traditional risk factor assessment. In experienced laboratories, this test can now be considered for further clarification of CHD risk assessment at the request of a physician."[44] The presence of abnormalities on the test would prompt aggressive medical therapies for primary prevention in individuals at intermediate risk, presumably lower targets for total and low-density lipoprotein cholesterol, ie, those used in secondary prevention. More recently, Bethesda Conference 34 recommended an individualized approach to noninvasive atherosclerosis testing "based on physician recommendation and referral, only after a careful consideration of known medical history and evaluation of major standard cardiovascular risk factors by office-based techniques."[45] In addition, carotid ultrasound is efficacious in detecting plaque in young individuals at risk for accelerated atherosclerosis caused by inflammatory conditions, such as systemic lupus erythematosus[33,34] and rheumatoid arthritis.[46] The extent to which more aggressive preventive therapy based on risk stratification by screening carotid ultrasound might favorably impact prognosis has not been examined.

FLOW-MEDIATED ENDOTHELIUM-DEPENDENT BRACHIAL ARTERY DILATION IN CARDIOVASCULAR RISK STRATIFICATION

During the past decade, a noninvasive technique called flow-mediated vasodilation (FMD) has evolved to evaluate vascular endothelial function in the brachial artery.[47-52] Blood pressure cuff occlusion of the brachial artery, and the subsequent shear stress produced by hyperemia on cuff release, provides a stimulus for release of nitric oxide from the endothelium. Brachial artery vasodilation can be imaged by ultrasound and quantified as an index of vasomotor function. BART studies have shown impaired vasodilatory responses in patients with coronary risk factors such as hypercholesterolemia,[53] hypertension,[54,55] smoking,[56] diabetes mellitus,[57] hyperhomocysteinemia,[58] and aging,[59] and in the presence of established CHD.[49] A close relationship between endothelial dysfunction in the coronary and brachial arteries has also been demonstrated.[60] Lipid-lowering therapy in those without[61] and with coronary artery disease,[62] antioxidants,[63] estrogen replacement,[64] and treatment with angiotensin-converting enzyme inhibition or receptor blockade[65] have each been shown to improve these responses.

Prognostic Use of FMD

A number of studies have examined the prognostic value of endothelial function assessment in predicting subsequent cardiovascular event rates. Studies have differed in the method (BART,[66-70] venous plethysmography with intrabrachial injection,[71,72] or intracoronary Doppler flow wire)[68,73,74]; cohort of patients studied (those with established atherosclerosis[75] vs those with risk factors for CVD)[69]; and statistical approach (comparison against traditional Framingham risk assessment models, univariate[76] vs multivariate modeling,[69,73-75,77] retrospective analysis,[78] and adjustment for degree of underlying coronary artery disease).[73] These studies have revealed that, in general, measures of endothelial function do not have additive prognostic use in patients at high risk.[77] The ability of FMD to provide prognostic information in individuals at intermediate or low risk, independent of more standard risk-profiling approaches, remains to be identified. Improvement in endothelial function in response to therapy predicts subsequent low event rate,[69] and the improvement of vascular reactivity during lipid-lowering treatment has been shown to be related to the reduction of C-reactive protein level–an indirect marker of chronic vessel wall inflammation and of insulin resistance.[79] Treatment modification may be guided by serial measurement of endothelial function. Interested readers are referred to an excellent review article that has previously been written on brachial artery reactivity.[80]

Examination Technique

Participant preparation. Study participants should fast for at least 8 to 12 hours before the study; they should be studied in a quiet, temperature-controlled room. All vasoactive medications should be withheld for at least 4 half-lives, if possible. In addition, participants should not exercise; ingest substances that might affect FMD such as caffeine, high-fat foods, and vitamin C; or use tobacco for at least 4 to 6 hours before the study. The investigator should be cognizant of the phase of a female participant's menstrual cycle.[81]

Image acquisition. The participant is positioned supine with the arm in a comfortable position for imaging the brachial artery. A sphygmomanometric cuff is placed either above the antecubital fossa or on the forearm (see "Upper arm or forearm cuff occlusion" below for a comparison of these two approaches). Brachial artery impulse is palpated superomedial to the antecubital fossa. To obtain the brachial artery images, the transducer may be moved superomedially slowly from the antecubital

fossa in a horizontal position with color flow Doppler turned on until the brachial artery and vein are seen in the transverse plane. The transducer is then rotated 90 degrees to perform imaging in the horizontal plane. Alternatively, the transducer may be placed in a vertical position along the lateral border of the biceps muscle, applying a firm constant pressure, and moved slowly medially until it slips in the medial groove of the biceps muscle, at which point a horizontal segment of brachial artery is seen at the bottom of the ultrasound screen. Minimal adjustment of the transducer allows the artery to be moved up toward the center of the image. The position of the transducer may be marked on the skin for future reference in the protocol. A segment with clear anterior and posterior intimal-lumen interfaces is selected for continuous 2-dimensional gray-scale imaging. Once the optimal artery image is obtained, the probe can be fixed in place using a stereotactic probe holder. The 2-dimensional image may be optimized using the depth function on the ultrasound system; alternatively, the zoom function may be used to magnify a selected segment of the brachial artery. Imaging depth and gain settings should be kept constant throughout the study. During image acquisition, anatomic landmarks such as veins and fascial planes are noted to help image the same segment of the artery throughout the study (Figure 4).

A pulsed wave Doppler recording is obtained from the midartery. Thereafter, arterial occlusion is created by cuff inflation to suprasystolic pressure. Typically, the cuff is inflated to at least 50 mm Hg above systolic pressure to occlude arterial inflow for 3 to 5 minutes. This causes ischemia and consequent dilation of downstream resistance vessels by autoregulatory mechanisms. Subsequent cuff deflation induces a brief high-flow state through the brachial artery (reactive hyperemia) to accommodate the dilated resistance vessels. The resulting increase in shear stress causes the brachial artery to dilate. The longitudinal image of the artery should be recorded continuously during cuff occlusion and for up to 2 minutes after cuff deflation. A midartery pulsed wave Doppler signal is obtained on immediate cuff release and no later than 15 seconds after cuff deflation to assess hyperemic velocity. The maximal increase in diameter occurs approximately 60 to 90 seconds after release of the occlusion cuff[79,80] (Figure 4).

Variations in technique. *Upper arm or forearm cuff occlusion.* The sphygmomanometer cuff may be placed above or below the antecubital fossa. When the cuff is placed on the upper part of the arm, reactive hyperemia typically elicits a greater percent change in diameter compared with that produced by the placement of the cuff on the forearm.[49,82-84] This may be caused by a greater flow stimulus resulting from recruitment of more resistance vessels, or possibly by direct effects of ischemia on the brachial artery. Compared with forearm cuff occlusion, upper arm occlusion is technically more challenging for accurate data acquisition because of collapse of the brachial artery, nonvisualization of color Doppler flow, and the shifts in artery and soft tissue that occur on cuff release. Upper arm occlusion may also be less comfortable for the patient. Regular blood pressure cuffs may make imaging difficult because of minimal space for imaging, especially when the arm is short or obese. A more narrow blood pressure cuff overcomes these problems and also allows instantaneous cuff deflation, facilitating use by a single operator. The change in brachial artery diameter after cuff release increases as the duration of cuff inflation increases from 30 seconds to 5 minutes. The change in diameter is similar after 5 and 10 minutes of occlusion. The percent change in artery diameter decreases as the baseline vessel diameter increases.[51,82,85]

Assessment of endothelium-independent vasodilation. At least 10 minutes of rest is needed after reactive hyperemia (ie, FMD) before another image is acquired to reflect the re-established baseline conditions. In most studies to date, an exogenous nitric oxide donor, such as a single high dose (0.4 mg) of nitroglycerin spray or sublingual tablet, has been given to determine the maximum obtainable vasodilator response, and to serve as a measure of endothelium-independent vasodilation reflecting vascular smooth-muscle function.[86] Peak vasodilation occurs 3 to 4 minutes after nitroglycerin administration; images should be continuously recorded during this time. Nitroglycerin should not be administered to individuals with clinically significant bradycardia or hypotension.

Measurement Technique

Analysis. Accurate analysis of brachial artery reactivity is highly dependent on the quality of ultrasound images obtained. Analysis may be performed by several methods. Software programs allow digitization of selected analog frames. Alternatively, a continuous digital recording may be obtained, or digital loops may be acquired at select time points. The boundaries for diameter measurements in the selected images (the lumen-intima or the media-adventitia interfaces) are measured manually with electronic calipers or, preferably, automatically using edge-detection software. Brachial artery diameter should be measured at the same time in the cardiac cycle using electrocardiographic gating during image acquisition (Figure 4). The onset of the R wave is used to identify end diastole, and the peak of the T wave reproducibly identifies end systole. For any given absolute change in the postflow stimulus diameter, a larger baseline diameter yields a smaller

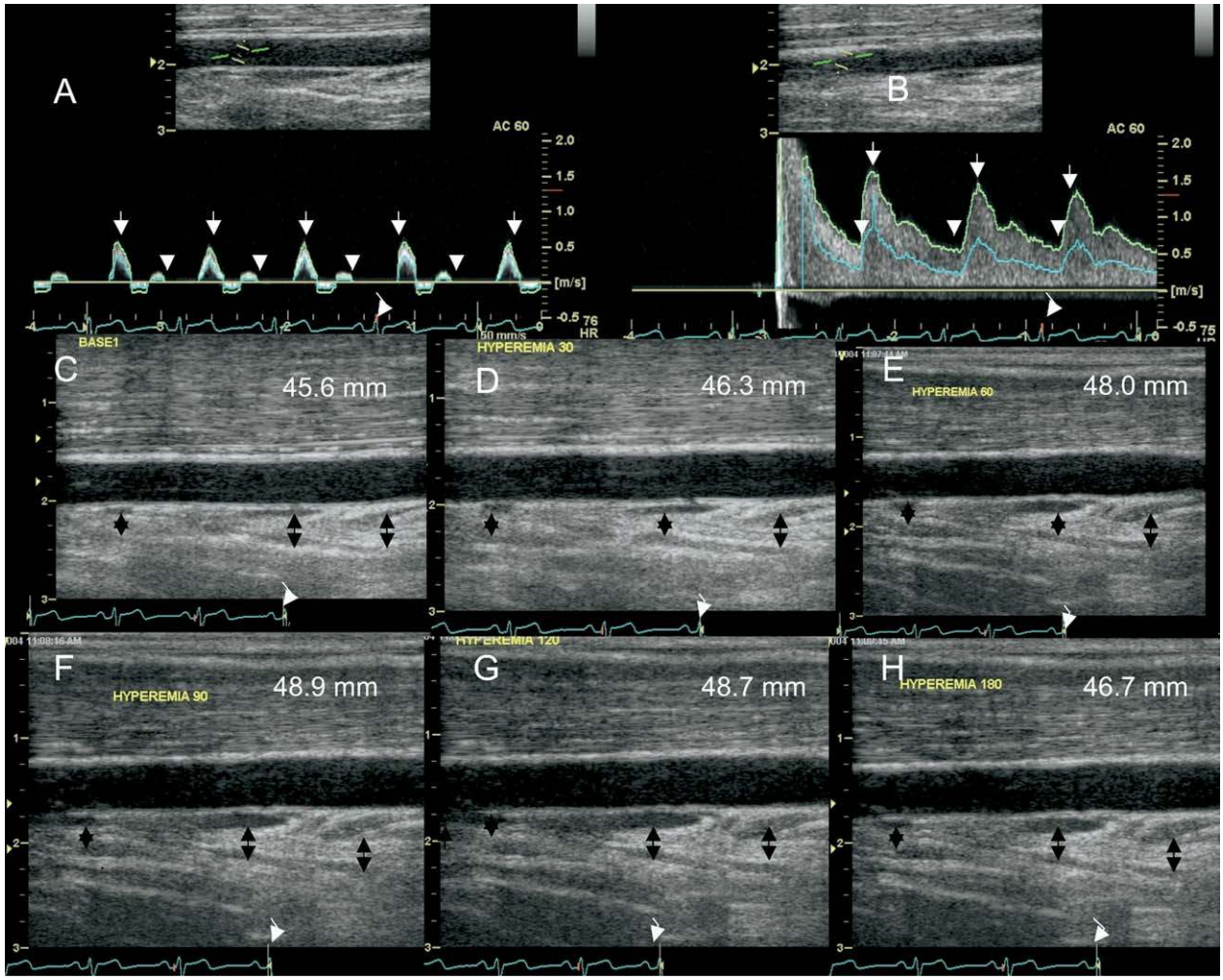

Figure 4 Ischemia-induced brachial artery (BA) reactivity test in healthy individual. Arm cuff was inflated at 200 mm Hg for 3 minutes. BA diameter responses (**C** to **H**) and pulsed wave (PW) Doppler velocity responses at baseline (**A**) and immediately on cuff release (**B**). Baseline 2-dimensional (2D) image of BA (**C**) with 30- (**D**), 60- (**E**), 90- (**F**), 120- (**G**), and 180- (**H**) second posthyperemia 2D images. Note increase in BA diameter in posthyperemia phase, compared with baseline, peaking at 90 milliseconds (**F**), and increase in flow and decrease in resistance immediately on cuff release (**B**) compared with baseline (**A**). Automated measurements of peak systolic velocity (*white arrow*), end-diastolic velocity (*white arrowhead*), peak velocity-time integral (*green tracing*), and mean velocity-time integral (*blue tracing*) are shown. PW Doppler assessment is made at 60-degree angle correction. BA diameter measurements are made at onset of QRS complex (**C** to **H**) (*angled white arrows*). Note fascial plane landmarks that are reproduced in each image (*double-sided black arrows*)

percent change. Thus, it is advisable to measure and report baseline diameter, absolute change, and percent change in diameter.

Limitations. Because the magnitude of brachial artery diameter change is a fraction of a millimeter, the technique requires extreme accuracy in methodology. Use of the zoom function may result in visualization of only a segment of brachial artery and make it difficult to track the brachial artery as it dilates on cuff release. In addition, use of zoom function may remove anatomic landmarks of fascial planes. A depth setting that allows visualization of surrounding fascial planes, and a long horizontal segment of brachial artery with clear definition of intima, is advisable. If measurement of augmentation of blood flow on cuff release is desirable, one must obtain pulsed wave Doppler data immediately on cuff release. Change in vessel anatomy on cuff release may make these data difficult to acquire. Dual live imaging of the artery and the pulsed wave Doppler signal is required so the Doppler cursor may follow the artery as it expands and shifts. In addition to errors related to improper technique, it is important to be aware of a host of factors that cause

intrinsic variability in FMD, including include mental or physical stress, recent intake of a meal,[87,88] medications including vitamins,[89] exogenous hormones,[90] time of day, cyclic changes related to the menstrual cycle in female participants,[91] age,[59] and body weight.[92]

Training and Quality Improvement

Despite its deceptively simple appearance, ultrasonographic assessment of brachial artery reactivity is technically challenging and has a significant learning curve. Any laboratory beginning to use this technique must ensure availability of duplex equipment with high-frequency linear-array transducers, and adequate sonographer training and a certification process. It is recommended that at least 100 supervised scans and measurements are performed before independent scanning and reading are attempted; 100 scans per year should be performed to maintain competency.[93]

Potential Indications for Risk Stratification

FMD has, thus far, remained a research technique, and its clinical use in determining the risk of future CVD events remains to be tested. The technique is skill- and labor-intensive and not yet easily used in routine clinical practice. Furthermore, interreader variability has led to difficulties in replicating data and quantifying the real magnitude of response. Standardization and improvement of the measurement technique are needed before this modality can become a routine clinical assessment of CVD risk. The technique is particularly well suited for study of the earliest stages of atherosclerosis in children and young adults, thus, providing maximal opportunity for prevention. However, additional prospective research is needed to demonstrate that this technique can truly add to clinical CVD risk prediction.

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