Measurement of Atherosclerotic Carotid Plaque Size In Vivo Using High Resolution Magnetic Resonance Imaging

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Background—Current imaging modalities, such as contrast angiography, accurately determine the degree of luminal narrowing but provide no direct information on plaque size. Magnetic resonance imaging (MRI), however, has potential for noninvasively determining arterial wall area (WA). This study was conducted to determine the accuracy of in vivo MRI for measuring the cross-sectional maximum wall area (MaxWA) of atherosclerotic carotid arteries in a group of patients undergoing carotid endarterectomy.

Methods and Results—Fourteen patients scheduled for carotid endarterectomy underwent preoperative carotid MRI using a custom-made phased-array coil. The plaques were excised en bloc and scanned using similar imaging parameters. MaxWA measurements from the ex vivo MRI were used as the reference standard and compared with MaxWA measurements from the corresponding in vivo MR study. Agreement between the in vivo and ex vivo measurement was analyzed using the Bland-Altman method. The paired in vivo and ex vivo MaxWA measurements strongly agreed: the mean difference (in vivo minus ex vivo) in MaxWA was 13.1±6.5 mm² for T1-weighted (T1W) imaging (mean MaxWA in vivo=94.7 mm², ex vivo=81.6 mm²) and 14.1±11.7 mm² for proton density–weighted (PDW) imaging (mean MaxWA in vivo=93.4 mm², ex vivo=79.3 mm²). Intraobserver and interobserver variability was small, with intraclass correlation coefficients ranging from 0.90 to 0.98.

Conclusions—MRI is highly accurate for in vivo measurement of artery WA in atherosclerotic carotid lesions. This imaging technique has potential application monitoring lesion size in studies examining plaque progression and/or regression. (Circulation. 1998;98:2666-2671.)

Key Words: atherosclerosis ■ magnetic resonance imaging ■ plaque ■ carotid arteries

Currently available methods for assessing the severity of atherosclerotic disease, such as contrast angiography and Doppler velocity waveform analysis, have been shown to accurately determine the degree of luminal stenosis. However, luminal narrowing is an indirect marker of plaque size and probably underestimates the atherosclerotic plaque burden. For example, Glagov et al demonstrated that vessels can sustain a large increase in atherosclerotic plaque mass without luminal narrowing as a result of compensatory enlargement of the adventitial boundary. Therefore, accurate determination of plaque size requires an imaging technique that can identify not only the boundary between the lumen and vessel wall, but also the outer border between the vessel wall and the surrounding tissues. Such a tool is necessary to define the relationship between total plaque burden and thromboembolic complications. Furthermore, accurate identification of atherosclerotic wall mass, rather than the degree of lumen narrowing, is needed to better understand the factors that result in plaque progression and regression, and to precisely determine the effectiveness of potential interventions such as aggressive lipid-lowering therapy.

Unfortunately, most currently available imaging techniques are unable to accurately identify the outer vessel wall boundary. Endovascular ultrasound has potential for determining arterial wall area (WA), but the invasive nature of the procedure makes it less suitable for serial studies. Although transcutaneous B-mode ultrasound has established utility measuring common carotid artery wall thickness as an indirect marker for atherosclerotic disease, the technique has had limited success in directly assessing carotid bifurcation plaque size and morphology. Because of acoustic anisotropic effects, the appearance of the arterial wall will change depending on the angle of insonation. Furthermore, the ability to visualize the plaque becomes difficult when calcium is present and when the lesion is located more distally in the internal carotid artery.

Several studies have shown that magnetic resonance imaging (MRI) is able to noninvasively image the arterial lumen and provide detailed information about vessel wall characteristics. With the advent of improved MR equipment and sophisticated surface coil design, an axial resolution of
250×250 μm can be achieved.3 Thus, MRI has potential for identifying both the lumen and adventitial boundary for determination of artery wall cross-sectional area and estimation of atherosclerotic plaque volume.

Optimally, WA measurements obtained from in vivo imaging should be validated against measurements conducted on serial histological sections of excised plaque specimens. However, there are many problems associated with morphometric measurements on histologic specimens.2 Disruption and alteration of the shape of the lesion can occur during excision, and shrinkage of the specimen may occur during fixation, dehydration, and decalcification.

Pan and his coworkers6 described a method using high-resolution ex vivo MRI of fresh atherosclerotic carotid endarterectomy specimens as the standard for validating lumen area measurements.6 The authors removed plaque specimens en bloc, thereby minimizing distortion of lesion morphology during excision. Shrinkage of the excised, fresh specimen was minimal: ex vivo MR measurements of lumen stenosis correlated closely to measurements of the atheroma wall and lumen following acrylic injection of the specimen under pressure (r=0.92).

In this study, serial cross-sectional MR images of atherosclerotic carotid plaques were obtained in a group of patients undergoing carotid endarterectomy. Serial cross-sectional MR images of the excised fresh endarterectomy specimens were obtained and used as the reference standard for comparison. The aims of this study were to determine the accuracy of high-resolution in vivo MRI for identifying cross-sectional WA in atherosclerotic carotid arteries and to examine intra- and inter-observer variability. Results from this study demonstrate that in vivo WA measurements strongly agree with those measured by ex vivo MRI. Furthermore, intraobserver and interobserver variability was minimal.

Methods

Study Population

Between July 1995 and July 1996, 14 consecutive patients scheduled to have carotid endarterectomy at the University of Washington Medical Center or the Puget Sound Veteran’s Affairs Medical Center were recruited for the study after giving informed consent. The consent forms and protocol were approved by each facility’s institutional review board. Before surgery, each patient underwent an MRI study of the extracranial carotid arteries. These in vivo MRI studies were conducted within 5 days before the surgery (mean=1.77 days), except in one case where there was a 23-day period between the in vivo and ex vivo study. This patient remained asymptomatic during the 23-day interval.

Protocol for MRI In Vivo and Ex Vivo

All the MR images were acquired on a 1.5-T whole body scanner (SIGNA, version 5.4, GE Medical Systems). To improve the signal-to-noise performance of the scanner, custom-designed bilateral phased-array carotid coils were used.7 The in vivo imaging protocol was optimized to visualize the arterial wall of the carotid bifurcation. The protocol included the following: (1) an axial 2D time-of-flight MR angiogram to determine the location of the carotid bifurcation, (2) an axial spinecho (SE) imaging sequence to acquire T1-weighted (T1W) images of the carotid artery wall, and (3) an axial fast spin-echo (FSE) imaging sequence to acquire proton density–weighted (PDW) images of the vessel wall. There was no slice gap between images, and the number of acquisitions was 2. The standard ECG gating technique available with the GE Signa package was used for PD-weighted in vivo imaging. The trigger delay varied from 30 to 800 ms (a function of spatial location and patient heart rate). Gating was not used for T1W imaging, because it did not significantly alter image quality in a previously published study.1 In both the SE and FSE sequences, signal from flowing blood in the carotid artery and in the adjacent internal jugular vein was suppressed via the application of radiofrequency saturation bands proximal and distal to the carotid lesion. To reduce subcutaneous fat signal and to avoid chemical shift artifacts, fat saturation technique was applied in both in vivo and ex vivo T1W and PDW images. The imaging parameters are summarized in Table 1. With this protocol, the in-plane resolution was 0.25×0.50 mm for T1W imaging and 0.50×0.50 mm for PDW imaging.

Plaque specimens were removed en bloc by scoring the adventitia with a scalpel without incising the intimal lesion. Thus, the morphology of the plaque and vessel lumen was preserved. After endarterectomy was performed, the remaining carotid artery wall was composed of adventitia and a thin layer of media and was ~0.5 mm thick. The endarterectomy specimen was placed immediately in nutrient cell culture medium (RPMI) to maintain physiological conditions during transport of the lesion. Furthermore, the ex vivo MR scans were conducted within 4 hours of surgery at body temperature (37°C) while submerged in RPMI. Plaque specimens acquired at the Puget Sound VA required across-town transportation at 4°C (15- to 30-minute transport time). Ex vivo MRI was conducted on the excised carotid endarterectomy specimens using the same whole body scanner. T1W SE and PDW FSE cross-sectional images of the specimens were obtained while placed in plastic container with a surface coil mounted. Imaging parameters are summarized in Table 1.

Vessel WA Measurement

Cross-sectional areas of the lumen and outer boundary of the vessel were measured using a custom interactive database language (IDL) program. Using this program, the lumen and the outer vessel wall boundaries were manually traced and cross-sectional areas were calculated. For in vivo images, the outer vessel wall boundary was defined as the vessel wall–soft tissue interface; for ex vivo images, the outer vessel wall boundary was defined as the vessel wall–RPMI interface. Wall area was defined as the area encircled by the outer vessel wall boundary minus lumen area. Figure 1 demonstrates a cross-sectional view of the common carotid artery on in vivo MRI (1A) and ex vivo MRI (1B). Corresponding outlines of the lumen and outer vessel wall boundaries are shown in Figure 1C and 1D.

The IDL program had the following features: (1) the original images were magnified (×4) and cropped to ensure optimal viewing, and (2) the signals of the sequential stack of images of the same subject were scaled so that the visual contrast of soft tissues
remained relatively constant. For each contrast weighting, each set of
cross-sectional images for a particular artery were analyzed sequen-
tially and the 14 cases were analyzed randomly. One measurement
was obtained for each determination. In vivo and ex vivo images
were measured in separate sessions to avoid potential bias.

Interobserver and Intraobserver Variability
To assess intraobserver variability, the WA 3 mm proximal to the
common carotid bifurcation was measured by a single reader, with
the second set of measurements performed 3 months after the first
set. To examine interobserver variability, a second reader determined
the WA at the same cross-sectional level and these measurements
were compared with the second set of measurements performed by
reader 1.

Data Analysis
The physical location of the carotid bifurcation in the images was
determined in both in vivo and ex vivo images as evidenced by the
conversion from a single lumen to dual lumens. Once this location
was defined, all other image locations were referenced to it in
millimeters: distal (cephalad) as positive and proximal (caudad) as
negative values. The comparisons between in vivo and ex vivo
images were conducted on the following quantitative measurements:
(1) maximum wall area (MaxWA) in common carotid artery or
internal carotid artery, (2) location of the MaxWA along the
longitudinal axis of the carotid artery, and (3) WA in the common
carotid artery 3 mm proximal to the common carotid bifurcation.

To determine the agreement in the WAs measured on in vivo and
ex vivo images, the statistical method described by Bland and
Altman\textsuperscript{9,10} for comparing paired data was used. Similarly, agreement
between the location of the MaxWA on in vivo and ex vivo images
was analyzed with the Bland-Altman method. To determine intraob-
server and interobserver variability, intraclass correlation coeffi-
cients and the lower limit of the 95% CIs were determined using
Statview 4.01 (Abacus Concepts, Inc) and methods described by
Armstrong et al.\textsuperscript{11}

Results
One patient was excluded from the analysis of the T1W
images because of poor image quality caused by patient
motion artifacts. A second patient was excluded from both the
T1W and PDW image analyses because the location of the
bifurcation could not be identified on the ex vivo images. As
noted above, the level of the bifurcation was defined as the
cross-section in which 2 lumina are first identified. In the
second excluded case, plaque from the external carotid artery
was not included in the specimen. Therefore, the level of the
bifurcation could not be identified on the ex vivo scan, and

Figure 1. Cross-sectional image of common carotid artery on in vivo (A) and ex vivo (B) T1-weighted MRI. The area of signal void
(arrow) adjacent to the lumen (L) represents a region of dense calcification, confirmed by histologic examination. Outline of lumen and
the carotid artery outer wall boundary (OWB) on corresponding in vivo (C) and ex vivo (D) T1-weighted MRI.
comparison to the corresponding in vivo cross-section could not be performed.

Comparison of In Vivo and Ex Vivo MaxWAs

The MaxWA values measured on in vivo and ex vivo images are summarized in Figure 2A and 2B. The range of MaxWAs was 50 to 125 mm² for T1W imaging and 48 to 116 mm² for PDW imaging. Mean MaxWA for in vivo and ex vivo T1W imaging was 94.7 and 81.6 mm², respectively, and 93.4 and 79.3 mm² for in vivo and ex vivo PDW imaging, respectively. Thus, the mean difference (in vivo minus ex vivo ±SD) in MaxWA was 13.1 ± 6.5 mm² (n=12) for T1W imaging (Figure 2A) and 14.1 ± 11.7 mm² (n=13) for PDW imaging (Figure 2B). Both of these positive mean values suggested that the MaxWA measured in vivo tended to be larger than that of ex vivo studies. However, using Bland and Altman’s analysis, we found that the paired in vivo and ex vivo MaxWA values strongly agreed, as evidenced by the small standard deviations. Furthermore, the variability for both T1W and PDW imaging was constant across a range of MaxWAs (Figure 2A and 2B).

Location of MaxWA in Common or Internal Carotid Artery

Figure 3 demonstrates that the MaxWA location identified on in vivo MRI (relative to the carotid bifurcation) closely agreed with the location determined on ex vivo MRI for T1W (3A) and PDW (3B) imaging. The mean±SD difference in location was 1.1±1.0 mm for T1W imaging and 0.7±1.7 mm for PDW imaging.

WA 3 mm Proximal to Common Carotid Artery Bifurcation

Figure 4A and 4B summarizes the WA measurements 3 mm proximal to the common carotid bifurcation. The range of MaxWAs was 43 to 118 mm² for T1W imaging and 46 to 117 mm² for PDW imaging. The level of agreement between in vivo and ex vivo MRI at this discrete level was similar to the MaxWA results. The mean±SD difference (in vivo minus ex vivo) in MaxWA was 12.3±8.3 mm² (n=13) for T1W imaging (Figure 4A) and 13.8±12.7 mm² (n=13) for PDW imaging (Figure 4B).

Intraobserver and Interobserver Variability

Intraclass correlation coefficients and the lower bound of the 95% CI for T1W and PDW in vivo and ex vivo imaging were
The high-resolution MRI technique described in this study differs from conventional x-ray angiography and MR angiography in that it highlights the vessel wall rather than the flowing blood. This feature is achieved through the use of spin echo–based imaging sequences with 2 flow saturation radiofrequency bands applied proximal and distal to the region of interest and with the use of custom made phased-array surface coils. Our results demonstrate that this technique provides a quantitative measure of plaque burden, as described by the MaxWA. In this study, we used vessel WA measurements from ex vivo MRI as our reference standard. This technique, described by Pan and coworkers, minimizes artifacts of tissue distortion and shrinkage that occur during fixation, dehydration, and histologic sectioning. The MaxWAs measured in vivo were marginally larger than corresponding MaxWA measurements ex vivo. This difference may be due to the fact that the in vivo measurement included the intimal lesion, media, and adventitia, and the ex vivo measurement included the intimal lesion and part of the media. The mean difference between the in vivo and ex vivo MaxWA measurement for T1W imaging was 13.11 mm². This area difference corresponds to a thickness of 0.4 mm, which is consistent with the thickness of the adventitia and residual media left behind after endarterectomy. (The average area circumscribed by the outer wall boundary on ex vivo T1W scanning was 92.3 mm² and has a radius of 5.4 mm. The ex vivo outer wall boundary area (92.3 mm²) plus the difference between the in vivo and ex vivo MaxWAs (13.1 mm²) is 105.4 mm² and has a radius of 5.8 mm. This difference (5.8–5.4 mm) is consistent with the thickness of the adventitia and residual media that comprise the artery wall following endarterectomy. Of note, the difference between in vivo and ex vivo lumen area measurements at corresponding sites was small [1.6±3.9 mm²]).

Overestimation of WA on in vivo scanning may also be explained by differing image resolution selected for the in vivo and ex vivo scans. The MRI parameters for in vivo and ex vivo scans were the same, except for a difference in the field of view and slice thickness (Table 1). For the in vivo scans, a larger field of view and slice thickness were selected to minimize total scan time. A larger pixel size on in vivo imaging could result in overestimation of maximal wall thickness and may contribute to the bias noted on the Bland-Altman analysis. However, given these parameters and the mean maximal wall areas measured in this study, the WA measurements in vivo would be overestimated by only 2.7 mm² in the worst-case scenario.

The variability of the measurement, or the “limits of agreement” described by Bland and Altman (±2 SD about the mean difference) was ±13.1 mm² for in vivo T1W imaging and ±23.3 mm² for PDW imaging. Although other diagnostic imaging techniques have not been analyzed in a similar fashion for determining WA, the variability of T1W MRI compares favorably to the interobserver and intraobserver variability reported for quantifying carotid artery lumen stenosis by digital subtraction angiography. For T1W imaging, the area represented by 2 SD corresponded to 13.8% of the average MaxWA, which was 94.7 mm². In a study involving 70 patients and 137 carotid arteries examined with multiplanar, selective intra-arterial digital subtraction angiography, Young and his coworkers noted that the variability between 2 observers reading that same arteriogram was ±22.4%. Within-reader variability was ±18.0%.

Variability between in vivo and ex vivo measurements may have occurred because similar cross-sections were not compared (longitudinal misregistration, or coregistration errors). However, location of the MaxWA, with respect to the carotid bifurcation on in vivo MRI, closely agreed with the location of the MaxWA on ex vivo MRI (difference, mean±SD=1.1±1.0 mm). Further reduction in coregistration errors would be achieved by acquisition of a larger number of axial images with thinner slice thickness, such as those done with 3D acquisition techniques. Other methods for reducing measurement variability include techniques to improve image resolution (smaller field of view, increasing the matrix). However, increasing the number of axial images and techniques to improve resolution increases total scan time, which may adversely effect image quality. We have found that the in vivo MRI quality was influenced primarily by patient motion and by the depth of the carotid artery. Motion artifacts have been significantly reduced by using a head holder and minimizing total scanning time. With regard to the issue of artery depth, the authors are investigating alternative surface-coil designs for deeper carotid arteries.

In summary, measurements of the MaxWA with high-resolution in vivo MRI strongly agreed with our reference standard, ex vivo measurements with MRI. Furthermore, within- and between-reader agreement was high, with T1W imaging yielding marginally better results for in vivo studies. This noninvasive imaging technique has potential application in studies examining the mechanisms involved in the growth of atherosclerotic carotid lesions and in clinical trials that require direct quantification of lesion size.

### TABLE 2. Intraclass Correlation Coefficients and Lower Bound of 95% CI for Intraobserver and Interobserver Variability, T1W and PDW, In Vivo and Ex Vivo Scanning

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