Structure of Plaque at Carotid Bifurcation
High-Resolution MRI With Histological Correlation

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Background and Purpose—The composition of carotid atherosclerosis was visualized by using 3D MRI at high resolution with 200-μm³ voxels. Magnetic resonance signal characteristics were correlated with plaque components, including collagenous cap, necrotic core, and calcification, to define resolution and other requirements for future clinical carotid MRI.

Methods—Twenty-one en bloc carotid endarterectomy specimens were imaged ex vivo by 3D gradient-echo MRI by using a 1.5-T clinical scanner with repetition time, echo time, and flip angle of 40 ms, 18 ms, and 20°, respectively. Plaques were placed in Gd-saline and imaged in a solenoid radiofrequency coil. For quantitative tissue-specific signal analysis, techniques were developed to match tissue sections analyzed by MRI and histology.

Results—Three-dimensional imaging resolved complex morphological features not visualized by density- or T₂-weighted 2D spin-echo imaging. The collagenous cap, necrotic core, and areas of focal calcification showed differing signal characteristics: mean contrast-to-noise ratio for cap versus underlying core was 20. The signal distributions for media and necrotic core overlapped but were resolvable in most specimens. The signal from thrombus was variable.

Conclusions—En bloc specimens provide a useful model for studying plaque MRI. By use of isotropic submillimeter resolution, the collagenous cap and underlying necrotic core typically can be distinguished, and calcification can be identified. Thrombus displays a wide variation in signal intensity. The techniques presented could facilitate future clinicohistological correlation studies for atherosclerotic plaque MRI. (Stroke. 2001;32:2516-2521.)

Key Words: arterial wall ♦ carotid arteries ♦ magnetic resonance imaging

In randomized trials, the selection of patients for carotid endarterectomy (CEA) has relied primarily on measurement of stenosis by x-ray angiography. Therefore, the potential therapeutic benefit of CEA depends critically on the adequacy of this parameter to measure stroke risk.¹⁻³ However, trial data show that with current diagnostic criteria, for every patient (in aggregate) in whom a stroke is prevented by surgery, several patients may undergo the procedure for no benefit, with significant hazard and expense.²⁻⁴,⁵ Several lines of evidence suggest that stroke risk is determined by plaque thromboembolic potential, which is only indirectly linked to the degree of stenosis.⁶⁻⁸ Consequently, if we are to stratify a patient’s stroke risk from carotid atherosclerosis, we must identify other plaque characteristics that more directly relate to thromboembolic potential.⁹⁻¹³

The morphological architecture of atherosclerotic plaques may become more complex with increasing plaque bulk and, therefore, degree of stenosis.¹⁴⁻¹⁵ However, carotid bifurcation lesions with equivalent degrees of stenosis can be highly heterogeneous in compositional architecture (Figure 1). Plaques dominated by fibrous tissue (Figure 1a) are presumed to be more stable than plaques containing a large bulk of friable atheroma with prominent hemorrhage (Figure 1c). Although this judgment seems reasonable, it has not been substantiated,¹⁶ because testing such a hypothesis requires techniques to visualize plaque morphology and composition and correlate these features with the patient’s clinical presentation.

Given sufficient spatial resolution, MRI can visualize carotid plaque structure and quantify differences in morphology and composition that are not observable with the use of other modalities. To date, magnetic resonance (MR)-based studies of atherosclerotic tissue primarily have been based on spectroscopy or 2D imaging.¹⁷⁻²¹ These techniques resolve tissue at a level of 1 to 2 mm in ≥1 direction, obscuring the submillimeter architecture of plaque by partial volume averaging.

To reduce partial volume averaging and define the tissue-specific signal characteristics of plaque components, we used 3D MRI to examine CEA specimens. This technique provided high-resolution images that were approximately isotropic and permitted detailed examination of plaque morphology.
and compositional elements. The MR images were correlated with tissue classification from histological images of these sections by using fiducial markers to coregister tissue sections.

**Subjects and Methods**

Atherosclerotic plaque from the carotid bifurcation was excised en bloc from patients undergoing CEA. Special care was taken at surgery to ensure that the plaques were extracted essentially intact. Specimens were transported in saline-filled containers stored at 4°C. Imaging generally was performed within 72 hours ex vivo.

Images were acquired with a 3D sequence, termed fast imaging with steady-state free precession (FISP), on a Siemens Magnetom 1.5-T scanner with 200-μm isotropic resolution by using repetition time, echo time, and flip angle of 40 ms, 18 ms, and 20°, respectively, and a field of view of 50 × 25 × 13 mm³ with 3 excitation averages. The imaging parameters were selected to provide the highest spatial resolution while retaining a high signal-to-noise ratio from fibrous tissue in acceptable imaging times (<15 minutes). Spin-echo, T2-weighted, 2-mm-thick 2D scans using 2500-ms repetition time, 80-ms echo time, and 200-μm in-plane resolution were also obtained from some specimens to illustrate the effect of using thicker slices. Specimens were imaged in normal saline treated with 1 part in 300 gadodiamide with the use of a custom solenoid coil. We imaged several plaques first in blood and then in treated saline, and the signal from different plaque components was unchanged. The presence of contrast agent in the saline allowed unambiguous identification of the lumen-plaque interface. In vivo, there are other mechanisms for differentiating the lumen from plaque, and the results of the present study are not limited to contrast-enhanced MR angiographic methods.

In preliminary experiments, plaques were imaged serially for up to 7 days with no discernible changes in MR signal with time. Presumably, the large molecular structure of Gd-DTPA allows minimal, if any, diffusion into plaque, particularly in the absence of perfusion pressure in the excised specimens. This is consistent with earlier studies of the effect of refrigerated storage on MR spectroscopic measurements of atheromatous abdominal aorta.

To obtain initial qualitative data relating the gross pathological appearance of carotid plaques with high-resolution MRI, 12 fresh CEA specimens were cross-sectioned into 3-mm blocks and photographed. Sectioning fresh plaques, which typically contained a mixture of both heavily calcified and friable components, frequently resulted in some tissue distortion, making coregistration with MR images challenging.

In 9 specimens, a fiducial-based marking system (see below) was applied, and the specimens were fixed in 10% formaldehyde and decalcified (Surgipath Decalcifier II, Surgipath Medical Instruments). Paraffin-embedded sections were stained with hematoxylin and eosin (H&E), Masson’s trichrome, and elastin van Gieson’s (EVG) stains to facilitate assessment of tissue composition. From the 9 specimens, 31 sections of histologically stained tissue were classified into the following types: fibrous cap, focal calcification, necrotic core, media, surface thrombus, and intraplaque hematoma. Additional single specimens were prepared by using the fiducial markers and examined undecalcified after embedding the tissue in methyl methacrylate and glycol methacrylate; then they were stained with the von Kossa stain for calcium salts.

Because the MRI data set has equal resolution in 3 orthogonal directions, images can be reconstructed in planes of arbitrary obliquity to the base data set. These multplanar reformations (MPRs) allowed matching of the angle of the histological sections as defined by the spatial registration process. Regions of interest (ROIs, identified as representing the fibrous cap and underlying necrotic core), the media, and focal calcification were selected on the histological image and evaluated on the matched MPR. For each plaque component, a single ROI was specified, and the signal intensity at the corresponding location on the MR image was measured. A contrast-to-noise ratio (CNR) was recorded as the difference of that signal from the background Gd-saline mixture in units of noise. Signal measurements were analyzed by using JMP (SAS Institute Inc) graphically (Figure 3) and compared with mean CNRs for each pair of tissue components (Table).

Standard normal distribution methods (t tests) were applied when distributions were approximately normal. Our data fulfilled the 3 assumptions necessary to use the t test: equal groups with similar SD, a sample size of at least 30, and independent data. When distributions were not assumed to be normal, a nonparametric test was applied (Wilcoxon signed-rank statistic).

For accurate matching of MR images to histological tissue sections, a fiducial marker–based system of registration was developed (Figure 1e). In this system, the specimen was glued onto a thin polyester sheet bearing fiducial notches. The fiducial sheet, plaque-affixed, was slid into grooves in a custom syringe fabricated by Stycast (Emerson and Cummings). Tissue blocks, 2.5 cm in thickness, were cut from the formalin-fixed decalcified specimen by using a multiblade arrangement with 6 parallel blades. The blades were oriented perpendicular to the polyester sheet and to the axis of the internal carotid artery. By use of calipers, the locations of the cuts, as noted in the sheet, were measured with respect to the fiducial notches. Histological microtome sections (5 μm) from the proximal surface of each block were taken for staining. MPR slices were prescribed relative to the fiducial notches in the polyester sheet, which were visible in images that were not reformatted. In this way,
200-μm-thick “virtual” slices were matched with the actual tissue sections.

Results
The 3D architecture of the plaques was often highly complex. Rapid changes in diameter along the lumen (Figure 2) and complex patterns consistent with fissuring or ulceration were observed repeatedly. As seen from the array of 200-μm-thick slices (Figure 2a), the substantial morphological changes in the longitudinal as well as the transverse directions were adequately resolved at the 200-μm level. The MRI acquisition parameters provided full 3D data sets of the entire surgical specimen. Selected levels are illustrated in Figure 2.

Signal values for various major plaque components are plotted in Figure 3. Focal calcifications had the lowest signal intensities, and collagenous cap tissue had the highest. The distributions of tissue signal differed from each other in central tendency but demonstrated some overlap, especially for necrotic core and media. Therefore, it is not possible to create a direct assignment of tissue type for each volume element on the basis of signal intensity alone. However, within individual sections, geometric cues that permitted the visual identification of regions of similar signal intensity corresponding to a given tissue type could be used, and determination of architectural composition was possible.

The Table lists the CNRs, which are based on signal statistics from the ROIs corresponding to Gd-saline reference, collagenous cap, necrotic core, media, and calcification.

**Collagenous Cap**
A collagenous region of relatively high signal was typically observable between the lumen and necrotic core (Figure 4).

**Necrotic Core**
The necrotic core subjacent to the collagenous cap generally emitted a lower signal (Figure 4).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Collagenous Cap</th>
<th>Media</th>
<th>Necrotic Core</th>
<th>Calcification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gd-saline</td>
<td>22±12 (29)</td>
<td>35±11 (23)</td>
<td>42±16 (27)</td>
<td>66±22 (21)</td>
</tr>
<tr>
<td>Collagenous Cap</td>
<td>12±12 (21)</td>
<td>20±12 (27)</td>
<td>43±17 (20)</td>
<td></td>
</tr>
<tr>
<td>Media</td>
<td></td>
<td>9±10 (20)</td>
<td>13±5 (16)</td>
<td></td>
</tr>
<tr>
<td>Necrotic core</td>
<td></td>
<td></td>
<td>23±12 (19)</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SD, with numbers in parentheses indicating number of comparisons. CNR was calculated from MPR images according to the following formula: $(S_x - S_y)/\sigma$ where $S_x$ and $S_y$ are the average signals from the compared components, and $\sigma$ is the signal standard deviation from a noise-only ROI from the slice in which the comparison was made. $P<0.0005$ that each CNR is different from zero (Wilcoxon signed-rank statistic).

**Figure 2.** a, Serial transverse MPRs with 200-μm in-plane resolution, 200-μm-thick slices, and zero interslice gap revealing rapid changes in plaque contour. b and c, Corresponding density (panel b) and T2-weighted (panel c) 2D spin-echo images with 2-mm slice thickness and substantial loss of detail. d, Averaged signal of the serial MPRs from panel a.

**Figure 3.** MRI signal distributions for collagenous cap (CC), focal calcification (Cal), media (Md), and necrotic core (NC). Values plotted are ROI means for specific tissues relative to Gd-saline reference signal, or $(s-g)/n$, where $s$, $g$, and $n$ are ROI mean magnitude signals for specific tissue, Gd-saline, and noise, respectively. Each plotted point is derived from a single MPR image matched to histological sections. Focal calcifications were excluded from ROIs for other tissues. Quantile boxes mark the 25th, 50th, and 75th percentiles; upper and lower cross bars mark the 10th and 90th percentiles. Central tendencies for cap, calcification, media, and core all differed significantly ($P<0.05$, by pairwise comparison of means by Student $t$ tests).
Media
Elastin-containing smooth muscle (black on EVG stain) generally, but not invariably, had a darker image than the fibrous cap (Figure 4).

Calcification
Calcifications produced marked signal loss. The effects can be demonstrated by comparing images of a fresh and decalcified specimen (Figure 4). There was relatively little effect on calcified areas from the formalin fixation alone, as shown by examination of en bloc specimens in 3 stages of preparation: fresh, fixed, and undecalcified versus fixed and decalcified.

Intraplaque Hemorrhage and Mural Thrombus
Blood accumulations produced variable signal. This was illustrated by 2 examples: in one example, an intraplaque blood collection emitted a bright signal (Figure 5a through 5f), and in the other, surface thrombus yielded a considerably lower signal (Figure 5g through 5k).

Discussion
Advanced atherosclerotic plaques of the carotid bifurcation typically display complex morphology. The size, shape, and location of the lumen, collagenous cap, necrotic core, and focal calcifications can vary substantially as one moves fractions of a millimeter longitudinally through the plaque. For a typical 2D scan, with slice thickness 2 mm, volume averaging severely limits the ability to faithfully visualize this complex architecture. The 3D imaging technique presented provided cubic voxels with 200-μm resolution. This reduced partial volume averaging (Figure 2) and facilitated matching MR images to histological sections for quantifying the signal characteristics of plaque components. The 3D MRI data presented show that isotropically submillimeter resolution will be required to reliably quantify plaque architectural components, including cap thickness, and thereby establish their role in promoting atheroembolic or thromboembolic events.

The sequence described in the present study, 3D-FISP, is similar to all standard MR angiographic sequences, apart from details of how flowing blood is accounted for in the in vivo setting. The sequence provides images with high contrast between intraluminal blood (which is bright from time-of-flight effects) and the plaque inner wall. Furthermore, the plaque outer wall is generally delineated by a hypointense rim corresponding to the muscular media.

Accurate coregistration of MR images with histological sections is necessary for reliable quantification of tissue-
specific signals because of the spatial variability of plaque architecture. But rigorous coregistration is difficult to achieve. The shape and integrity of a specimen are degraded in histological preparation: decalcifying and fixing cause tissue shrinkage; cutting the plaque into blocks, slicing 5-μm sections, and mounting onto glass slides may all distort plaque architecture. In addition, standard techniques of histological preparation and microscopic examination do not provide accurate volumetric data unless painstaking efforts are applied to the preparation and processing of the tissue.15

The fiducial marker–based method used in this project operates independently of the MR appearance of the plaques. The method eliminates the need for searching through a 3D MR data set on a trial-and-error basis to generate MPRs to match given histological sections. Distortion of histological sections was still seen in our specimens and occasionally limited tissue identification.

Specimens were immersed in Gd-treated saline during imaging to provide artificial contrast between the plaque and its ex vivo environment. This facilitated visualization of luminal morphology, but our mean CNR of >20 between saline and collagenous cap cannot be extrapolated to clinical imaging. Obtaining contrast between plaque and blood in vivo is a problem but can be approached with flow-imaging techniques that include intravascular Gd-chelate administration or sequences that eliminate signal from flowing blood.

The levels of intertissue contrast in the Table, if they can be replicated in vivo, may be sufficient for clinical exploitation. In several MRI applications, reader preference analysis25 has suggested that CNRs of ~20 (the mean CNR for collagenous cap with respect to necrotic core in the present data) were optimal in the sense that further gains would be better used by decreasing voxel size. In view of the geometric complexity that we have described for carotid disease, these findings suggest that high-resolution (3D) sequences could be used, even at the expense of some CNR available with 2D spin-echo sequences.

Collagenous cap was associated with high signal in the 3D data sets, as had previously been described with the use of 2D density and T2-weighted methods.18,26 Reasons for high collagenous cap signal compared with necrotic core have been discussed.18 The 3D-FISP sequence provides images with mixed weighting, because it is influenced by T1, T2*, and density values. Nevertheless, this sequence provides a contrast between different tissue components that is as high as the contrast obtained with spin-echo sequences but with a 10-fold reduction in slice thickness (Figure 2). Bassiouney et al27 have provided evidence suggesting that the minimum thickness of the collagenous cap is related to the risk of a ipsilateral neurological event. They found that the mean distance between necrotic core and lumen was 500 μm in asymptomatic subjects (n=40) and close to 300 μm in symptomatic subjects (n=59). Resolution and contrast requirements for accurately determining these parameters are exacting and currently difficult to achieve in vivo. However, minimum thickness may not prove to be the only prognostic feature of the cap. Features more easily detected by MRI, such as large discontinuities of collagenous cap signal (Figure 5k), also may be indicative of plaque rupture and instability.

Not all collagenous tissue could be correctly classified solely on the basis of signal level. Signal variations may reflect variations in composition of collagenous tissue, particularly in the proportion of water. Signal loss from calcification located along the boundary between fibrous cap and necrotic core could obscure part of the fibrous cap. The comparison of MR images of fresh, fixed, and decalcified specimens suggests that the dark rims along the boundary between collagenous tissue and necrotic core are commonly associated with calcification. Because thrombus can vary from bright (methemoglobin) to dark (hemosiderin), it too can approximate the signal of the fibrous cap.

As expected, the presence of calcification in plaque tissue resulted in strongly lowered signal intensity. This was confirmed by the presence of signal voids at the locations of local calcification, as revealed on H&E and von Kossa stains of methyl methacrylate– and glycol methacrylate–embedded tissue, and by comparing images of fresh and decalcified tissue. Standard histological processing of calcified plaques involves decalcification by an acidic solution before paraffin embedding and micromotting. It is likely that traces of microcalcification are removed in the processing and leave no observable remnants. As described by Yuan et al28 (who used calcium-specific stains of fresh frozen undecalcified sections), diffuse calcification is commonly present in lipid-rich regions of plaques. The presence of stippled calcification will influence MR signal in such regions and may explain some signal variability observed in the lipid cores. This will be an important area for future investigation.

In sequences with heavy T2* weighting, areas of calcification are expected to produce susceptibility artifacts secondary to distortion of the local magnetic field. These could exaggerate the size of calcifications. Although a full discussion is beyond the scope of the present study, we have examined those effects with micro-CT methods, and susceptibility effects appear to be negligible for the sequence reported in the present study.

Accurate identification of thrombus remains the most challenging issue in plaque composition analysis by MRI. A bright signal is suggestive of methemoglobin in a relatively fresh hematoma, whereas a low signal may indicate hemosiderin in an older thrombus or hematoma (Figure 5). In addition to in vivo progression from formation of methemoglobin and, subsequently, hemosiderin, MR signal from blood products could be altered ex vivo by changes in hemoglobin oxygenation state. Oxygen tension was not controlled in these experiments but may be required in the future to identify reliably thrombus and hematoma.

The present study was initiated to establish the ability of 3D MRI to characterize the composition of atherosclerotic plaque. The method improves the identification of plaque components by reducing partial voluming effects. Our study establishes spatial resolution limits that are sufficient for identification of the major plaque components, although the necessary spatial resolution will still likely depend on the specific clinical question. The full value of this imaging method will be realized only when it can be applied in vivo. Apart from the use of a dedicated small-volume coil, the scanner hardware and software used for imaging the speci-
mens was the same as used in routine clinical practice. The imaging time, although lengthy, was not prohibitive. We have conducted preliminary studies in vivo by using the same sequence as used in the present study. Those results suggest that in vivo plaque characterization is achievable (as can be noted in Figure 5e and 5f) but that specialized pulse sequences and improvements in coil technology will be necessary to provide the resolution needed to consistently differentiate key components of plaque. The ability to identify plaque components will permit studies that could shed light on how plaque composition modifies the risk of neurological events over and above that conferred by geometric descriptors, such as diameter stenosis.

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