The Diagnostic Accuracy of Ex Vivo MRI for Human Atherosclerotic Plaque Characterization

Meir Shinnar, John T. Fallon, Suzanne Wehrli, Michael Levin, Dolcine Dalmacy, Zahi A. Fayad, Juan J. Badimon, Martin Harrington, Elizabeth Harrington, Valentin Fuster

Abstract—Recent evidence indicates that the type of atherosclerotic plaque, rather than the degree of obstruction to flow, is an important determinant of the risk of cardiovascular complications. In previous work, the feasibility of using MRI for the characterization of plaque components was shown. This study extends the previous work to all the plaque components and shows the accuracy of this method. Twenty-two human carotid endarterectomy specimens underwent ex vivo MRI and histopathological examination. Sixty-six cross sections were matched between MRI and histopathology. In each cross section, the presence or absence of plaque components were prospectively identified on the MRI images. The overall sensitivity and specificity for each tissue component were very high. Calcification and fibrocellular tissue were readily identified. Lipid core was also identifiable. However, thrombus was the plaque component for which MRI had the lowest sensitivity. A semiautomated algorithm was created to identify all major atherosclerotic plaque components. MRI can characterize carotid artery plaques with a high level of sensitivity and specificity. Application of these results in the clinical setting may be feasible in the near future. (Arterioscler Thromb Vasc Biol. 1999;19:2756-2761.)

Key Words: MRI ▪ atherosclerosis ▪ carotid endarterectomy

Disruption of atherosclerotic plaques is the most frequent underlying cause of the unpredictable onset of acute thromboembolic vascular events including sudden death, myocardial infarction, unstable angina, stroke, transient cerebral ischemia, and peripheral thromboemboli. Although clinical risk factors for atherosclerosis help predict risk of these events, identification of patients with plaques vulnerable to disruption is not possible by angiography that only visualizes the lumen. There is therefore a need for an in vivo noninvasive method for characterizing atherosclerotic plaques and identifying the “vulnerable” plaque.

Previous work has shown that MRI can characterize both ex vivo3-7 and in vivo8-11 the composition of human atherosclerotic plaques. However, the sensitivity and specificity of MRI have not been determined.

This study reports the development of high-resolution MRI criteria for the ex vivo tissue characterization of human carotid atherosclerotic plaques and their sensitivity and specificity in comparison with histopathology. Using these criteria, a semiautomatic segmentation algorithm is developed for characterizing the constituents of an atherosclerotic plaque.

Methods

Specimens

Human carotid endarterectomy specimens were studied. Specimens were obtained fresh and intact from the operating room, washed in phosphate buffered saline, grossly described, and samples taken for routine surgical pathology. The remaining 1- to 2-cm-long segments were flash frozen at −80°C until imaged. On the day of imaging, the specimens were placed in saline and slowly warmed to 37°C in a water bath. The artery was placed in either a 10- or 12-mm MR tube (Wilmad Glass) using the smallest possible tube for a given specimen. Care was taken to remove any air bubbles. Previous studies have shown no change in T1 and T2 of atheromatous plaques under these conditions of freezing and rewarmed.5

MRI

Specimens were imaged on a Bruker AM 400 wide bore (89 mm) 9.4T magnet with a gradient insert (ID 75 mm, maximal strength 50G/cm), controlled by an ASPECT 3000 spectrometer. The tube with the specimen was placed in either a 10- or 12-mm radiofrequency probe and positioned inside the magnet. During imaging, specimens were maintained at 37°C. Before imaging each specimen, the magnetic field was made homogeneous (shimmed).12 After initial scout images, cross-sectional spin echo images of the plaque were obtained. Four acquisitions (NEX) were averaged for each image. The field of view was 12.4 mm. The images were acquired as a 256×256 pixel matrix for an in-plane resolution of 48.3 μ. Images were obtained every 1 mm with a slice thickness of 500 μ and an interslice distance of 500 μ. For each cross section, 6 different spin echo images, using different repetition times (TR) and echo times (TE), were obtained (Table 1). A spin echo diffusion-weighted image13,14 was also obtained for each cross section. The resolution and NEX were the same as for the spin echo images. The TR was 2000 ms and TE 30 ms. Diffusion was measured in 1 axial direction. The diffusion parameters were a gradient strength of 16.67 gauss/cm,
duration of the gradient pulses (Δ) of 9.69 ms, and a separation of the gradient pulses (ΔΔ) of 12.69 ms, resulting in a diffusion weighting (b) of 1766 sec/mm². The signal intensity of the diffusion-weighted image was reduced from the signal intensity of the spin echo image with the same TR and TE by e⁻¹⁰, where D is the diffusion coefficient of water. For comparison, in most clinical diffusion-weighted imaging sequences, b is approximately 1000 sec/mm². A gradient strength of 10 gauss/cm (b=636 sec/mm²) was also used for comparison in some samples. The effect of a fat suppression pulse, calibrated using the spectrum of the entire sample, was tested in comparison in some samples. The effect of a fat suppression pulse, weighted imaging sequences, b is approximately 1000 sec/mm².

### Histopathology

At the conclusion of the MRI experiment, specimens were fixed in 10% buffered formalin. Specimens were decalcified in 1% formic acid, serially cross sectioned every 2 mm, embedded in paraffin, sectioned at 5 μm, and stained with a combined Mason elastic (CME) and hematoxylin and eosin (H&E).

### Matching of MRI and Histopathology

The MRI images and the histopathological slides were matched using the known location and distance between MRI and between histopathological cross sections. The gross morphology of each MRI cross section was then used to optimize the match with a corresponding histopathology cross section. We did not account for shrinkage of the specimen, as it can vary across specimens. However, morphologically, there seemed to be a good match between the corresponding sections. We tried to match as many images and slides per specimen as possible.

Each histopathological specimen was divided into 4 quadrants, trying to put components such as lipid-rich necrotic core and thrombus near the middle of a quadrant. The matched MRI images were then divided into corresponding quadrants.

### Image Processing and Analysis

MRI cross sections were transferred from the Bruker spectrometer to a Macintosh workstation and analyzed with IDL (RSI). The 7 data sets collected for each cross section were converted and displayed by normalization and scaling to an 8-bit gray scale. In addition, 3 parametric images (T2, T1, and diffusion coefficient) were derived from the original MRI data sets. Inverse T2 was calculated from linear regression of the log of the data sets with TR=2000 ms, TE=13, 30, and 50 ms against the echo times and displayed as the T2 value. T1 was calculated by a nonlinear fitting routine using data sets with TE=13 ms, TR=300, 700, 1000, and 2000 ms.

MRI criteria were developed for each plaque component using the first 5 specimens (Table 2). The developed criteria were applied by one observer (M.S.) blinded to the histopathology to determine the presence or absence of the following plaque components in each of the matched MRI cross sections: 1) calcification, 2) fibrocellular tissue, 3) fibrocellular tissue with extracellular lipid, 4) lipid-rich core, 5) thrombus, and 6) a fibrous cap, defined as a region of fibrocellular tissue with or without calcification overlaying a lipid-rich core. On the basis of our histopathological slides, it was not possible to date the thrombus, and we included both organized and unorganized thrombi. The quadrants in which each component was located were noted.

At a separate time, the matching histopathological cross sections were classified using standard histopathological criteria. Each of the plaque components can be reliably identified from a CME stained slide. Sudan black staining is not necessary to identify the lipid core, which is readily distinguishable from thrombus. This matching was done without knowledge of the MRI data. The quadrants in which a given component was located were noted. Then the MRI and histopathological classification of matched sections were cross tabulated.

### Results

Twenty-two human carotid endarterectomy specimens were imaged. In these specimens, the MRI and histopathology of 66 different cross sections were matched. The number of matched cross sections per artery averaged 3 and ranged from 1 to 4. Given the advanced nature of plaques that require endarterectomy, most plaques were complex (AHA type VI) and had most or all plaque components.

### MRI Criteria

For the first 5 samples, we used previously published criteria, which emphasize the use of T2-weighted images (TE 50 ms) to identify lipid core. However, to classify the plaque components accurately, these criteria were modified in 2 different ways.

1) Identification of thrombus. T2-weighted images did not allow for the accurate detection of thrombus. Initial results on the first specimens showed that diffusion-weighted MRI does allow for the detection of thrombus on the basis of the restriction of diffusion of water in the clot.

2) Distinguishing fibrocellular tissue with lipid from lipid core. T2-weighted images did not distinguish lipid-rich core from fibrocellular areas containing lipid. However, the use of 2 different echo times (30 and 50 ms) did distinguish the 2 (Figure 1).

The use of fat suppression had minimal effect on the image (Figure 2). Table 2 summarizes the criteria used for classifying plaque components.

### Table 1. MRI Spin Echo Parameters

<table>
<thead>
<tr>
<th>Image Type</th>
<th>TR  (ms)</th>
<th>TE  (ms)</th>
<th>Effect on Image</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proton density</td>
<td>2000</td>
<td>13</td>
<td>Proton density</td>
</tr>
<tr>
<td>T1</td>
<td>1000</td>
<td>13</td>
<td>Slight T1 weighting</td>
</tr>
<tr>
<td>Partial T2</td>
<td>300</td>
<td>13</td>
<td>Heavily T1 weighted</td>
</tr>
<tr>
<td>T2</td>
<td>2000</td>
<td>30</td>
<td>Moderate T2 weighting</td>
</tr>
<tr>
<td>T2</td>
<td>2000</td>
<td>50</td>
<td>T2 weighted</td>
</tr>
</tbody>
</table>

### Table 2. MRI Criteria for Identification of Plaque Components

<table>
<thead>
<tr>
<th>Component</th>
<th>Proton Density (TR=13 ms)</th>
<th>T2 (TE=30 ms)</th>
<th>Partial T2 (TE=30 ms)</th>
<th>Diffusion Weighted (TE=30 ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>Dark</td>
<td>Dark</td>
<td>Dark</td>
<td>Dark</td>
</tr>
<tr>
<td>Fibrocellular</td>
<td>Light</td>
<td>Light+++</td>
<td>Light§</td>
<td>Dark</td>
</tr>
<tr>
<td>Fibrocellular+lipid</td>
<td>Light+++</td>
<td>Light§</td>
<td>Light§</td>
<td>Dark</td>
</tr>
<tr>
<td>Lipid rich core</td>
<td>Light+++</td>
<td>Light§</td>
<td>Light§</td>
<td>Dark</td>
</tr>
<tr>
<td>Thrombus</td>
<td>Light</td>
<td>Variable</td>
<td>Light</td>
<td>Light§</td>
</tr>
</tbody>
</table>

*Less than 4 times noise level; §greater than 1/3 the water signal; ††Less than 1/5 the water signal.
Image Segmentation

The MRI criteria allowed for the development of a semiautomated segmentation routine (Table 3; Figures 1 and 3). First, the proton density image was examined for calcification, ie, pixels with signal <4× background noise. Second, lipid core was identified by a T2<17 ms on the parametric T2 image. Third, thrombus was detected by a diffusion coefficient of <3×10⁻⁶ cm²/sec. Fibrocellular areas containing lipid were identified by a T2 between 17 and 20 ms, and fibrocellular areas without lipid were characterized by a T2>20 ms on the parametric T2 image. T1 values were helpful in distinguishing fibrocellular areas from the bathing saline solution.

MRI Images

Figures 1 and 3 show the MRI and derived images, the histopathology, and the segmentation image for 2 representative carotid endarterectomy cross sections, respectively. The MRI images show different levels of contrast among the various plaque components. However, the proton density and the T1-weighted images show similar levels of contrast. Figure 4 shows 8 MRI cross sections, 1 mm apart, through the entire human carotid endarterectomy specimen used for Figure 1. The rapid change in the plaque composition over small distances is seen.

Sensitivity and Specificity

Table 4 summarizes results of the sensitivity and specificity testing of the final MRI criteria used for evaluating all 66 cross sections. The overall sensitivity and specificity are very high. Thrombus was the plaque component for which MRI had the lowest sensitivity. On review of the images, most thrombi that were not identified by MRI were adjacent to calcified tissue making the area dark on all images. The appearance of thrombus was sufficiently variable that it could not be reliably identified without using the diffusion-weighted image.

Location of Components

For the purposes of this study, we did not require that the components be identified by MRI and those identified by histopathology be at the identical location in the image to conclude that the 2 methods agreed. This was because of methodological issues of identifying the same location with these different techniques. As a measure of correspondence, we divided each MRI and histopathological specimen into quadrants. The components identified on the MRI images and the histopathology appeared in corresponding quadrants.

Discussion

Our results show that MRI tissue characterization of complex human atherosclerotic plaques can be accomplished ex vivo with a high degree of sensitivity and specificity. This investigation validates and extends previous studies. This study demonstrates that MRI can identify and characterize all major atherosclerotic plaque components and defines the MRI sequences and criteria needed for ex vivo plaque characterization. In vivo application of the MRI criteria may allow for the noninvasive, prospective identification of patients with plaques at high risk of clinical events and for the testing and institution of appropriate anti-atherosclerotic therapy.
There are several methodological issues and concerns that arise from this study. A previous study suggested that a T2-weighted image and an image to look at calcifications, either T1-weighted or proton density-weighted, would allow for the full characterization of atherosclerotic plaques. This suggests that full characterization can be done with a double echo sequence, obtaining both a T2-weighted image and a proton density-weighted image. This study shows that for accurate, full classification, the following MRI images are required: 1) proton density image, 2) T2-weighted (TE=50 ms) image, 3) partially T2-weighted (TE=30 ms) image, and 4) a diffusion-weighted image.

The proton density of water is a main determinant of signal intensity in MRI images. Calcified tissues, which have very little water, appear dark on all MRI images. However, fibrocellular tissue and thrombus may also be relatively dark on proton density images. Specifically, these plaque components appear darker on T2-weighted images than their actual T2 would suggest. One way to compensate for this problem is the use of parametric images. Actual T2, calculated from the signal intensity at 3 different TEs, allows for separating the effects of proton density from relaxation. However, calculating T2 from only 3 points is prone to error. Because these components, ie, fibrocellular and thrombus, may have relatively low signal, the low signal to noise creates even greater errors in the estimation of T2 parameters. However, in spite of the limitations of calculated relaxation parameters, as is evident by the noise in the images of the relaxation parameters (Figures 1 and 3), they were still useful in the semiautomatic segmentation routine. The application of techniques for the rapid determination of T2 of tissues may prove clinically useful in this regard.

It has been suggested that a T2-weighted image alone can reliably identify the necrotic core of an atherosclerotic plaque. This finding needs to be qualified because fibrocellular areas with extracellular lipid are also black on T2-weighted images (see Figure 1). This study demonstrates that use of a partially T2-weighted (TE=30 ms) image helps to differentiate between these 2 components (Table 2).

This study confirms preliminary data that diffusion-weighted MRI is a good technique for identifying thrombus and hemorrhage in plaque. Thrombus appears as a bright area in diffusion-weighted images. However, areas of thrombus adjacent to foci of calcification gave signals so low that thrombus did not appear bright on the diffusion-weighted sequence and was thus lost to identification. Complicating the identification of thrombus is the suggestion that acute thrombus may not have this characteristic bright appearance on diffusion-weighted images.

In some plaques, the diffusion-weighted image showed a bright spot that did not correspond to thrombus (Figure 3). These occurred in areas where the T2-weighted images were relatively bright. The parametric image of the diffusion coefficient was used in any doubtful cases.

It might be difficult to implement the precise diffusion sequence used in this article on a clinical scanner because of the extremely strong gradients used. Furthermore, ex vivo samples may differ in their diffusion properties from in vivo samples, where the intact cell wall presents a barrier to diffusion. However, imaging with a diffusion weighting even stronger than our partially diffusion-weighted images are obtained routinely, suggesting that one can obtain diffusion-weighted images of plaques in vivo. There are still significant problems in obtaining high-resolution diffusion images in vivo, because of the problems of motion and low signal to noise of these images.

In this study, T1-weighted images added little additional information beyond that available from the proton density and T2-weighted images. For the plaque components of interest, the tissue contrast of the T1-weighted and the proton density–weighted images were similar.

Fat suppression pulses had little impact on the images obtained in this ex vivo study. This confirms previous results, which showed that lipids constitutes only a small portion (~11%) of the MRI signal from the lipid-rich atheromatous core. However, fat suppression is necessary for in vivo imaging because periartrial fat can induce chemical shift artifacts.

<table>
<thead>
<tr>
<th>Component</th>
<th>Proton Density</th>
<th>T2 (ms)</th>
<th>Diffusion ((\text{cm}^2/\text{sec}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>Dark*</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Fibrocellular</td>
<td>Light†</td>
<td>&gt;20</td>
<td>&gt;0.3×10^{-5}</td>
</tr>
<tr>
<td>Fibrocellular + lipid</td>
<td>Light†</td>
<td>&gt;17−20</td>
<td>&gt;0.3×10^{-5}</td>
</tr>
<tr>
<td>Lipid rich core</td>
<td>Light†</td>
<td>&lt;17</td>
<td>...</td>
</tr>
<tr>
<td>Thrombus</td>
<td>Light†</td>
<td>&gt;17</td>
<td>&lt;0.3×10^{-5}</td>
</tr>
</tbody>
</table>

*Less than 4 times noise level; †greater than 4 times noise level.
Image Segmentation
The image segmentation routine developed in this study requires minimal user input. Its ability to segment the image and correlate with histopathology is further proof of the ability of MRI to identify different plaque components (Figures 1 and 3). An automated analysis program has several advantages: it eliminates observer bias; it allows for consistent, objective analysis across many samples; and segmentation allows one to display and summarize information culled from several different MRI images of the same tissue section. Similar segmentation routines should be applicable to in vivo MRI not only for arterial plaques but also for other tissues.

Limitations
Specimens were obtained from patients undergoing carotid endarterectomy. Thus the incidence of pathology was high and the atherosclerotic lesions were advanced. All samples, for example, had some calcification. Table 4 shows that the 95% confidence limits for the specificity of some of the components is very broad. Therefore, these results need to be substantiated by examination of specimens with less severe atherosclerotic plaques, not just those that come to endarterectomy. A representative study may therefore require an autopsy study, which has many other significant limitations, such as the degradation of the sample.
Ideally, endarterectomy specimens should be studied fresh from the body and maintained at 37°C because many techniques of tissue preservation are known to change MRI characteristics. For example, fixation in formalin, used in some studies, is known to change the relaxation parameters (T1 and T2). For logistical reasons, the specimens used in this study were frozen and then rewarmed to 37°C before imaging. Preliminary data suggests that there is no significant change in the MRI parameters under these conditions. However, the lipids in an atherosclerotic plaque are known to undergo a partially irreversible phase transition when cooled. It is unclear whether or not this phase transition of the lipids in the specimens affected the MRI appearance of the plaque.

The classification of the different components is not done with completely independent measurements, as the same images are used to classify all the components. However, this lack of independence is not a significant problem and may even accentuate any errors. Thus, if a lipid-rich fibrocellular area is misclassified as lipid-rich core, both sensitivity of the technique for lipid-rich fibrocellular areas and the specificity for lipid-rich core are decreased. Therefore, the lack of independence may magnify the effect of any mistakes.

Finally, the MRI criteria derived from this study may not apply directly to the clinical setting typically done in a 1.5 T magnet. Because T1 and T2 change with the field strength, further study is needed to determine the appropriate TE for in vivo applications.

We chose 9.4 T for several reasons. First, the previous studies on T2-weighted imaging were done at 9.4 T. Second, we thought we would need the high resolution and signal to noise obtainable at 9.4 T. Our images were obtained with a 48-μ in-plane resolution. We do not yet know the resolution needed for plaque characterization in vivo. However, in our study, we ignored plaque components that were only 1 or 2 pixels large, suggesting that we may not need this high resolution. Third, we lacked the appropriate instrumentation for doing temperature controlled, high-resolution imaging on our clinical scanner.

The characterization of the atherosclerotic plaque is assuming greater importance in determining the risk of cardiovascular events. This study shows that by using multiple MRI sequences and images, atherosclerotic plaques can be completely characterized. Furthermore, the sensitivity and specificity are high. The next step is to prospectively use these sequences in vivo for the characterization of atherosclerotic plaques in patients. This may eventually allow for the noninvasive evaluation of the risk of clinical cardiovascular events in the individual patient.

### Acknowledgments

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### References

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