In Vivo Accuracy of Multispectral Magnetic Resonance Imaging for Identifying Lipid-Rich Necrotic Cores and Intraplaque Hemorrhage in Advanced Human Carotid Plaques

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Background—High-resolution MRI has been shown to be capable of identifying plaque constituents, such as the necrotic core and intraplaque hemorrhage, in human carotid atherosclerosis. The purpose of this study was to evaluate differential contrast-weighted images, specifically a multispectral MR technique, to improve the accuracy of identifying the lipid-rich necrotic core and acute intraplaque hemorrhage in vivo.

Methods and Results—Eighteen patients scheduled for carotid endarterectomy underwent a preoperative carotid MRI examination in a 1.5-T GE Signa scanner using a protocol that generated 4 contrast weightings (T1, T2, proton density, and 3D time of flight). MR images of the vessel wall were examined for the presence of a lipid-rich necrotic core and/or intraplaque hemorrhage. Ninety cross sections were compared with matched histological sections of the excised specimen in a double-blinded fashion. Overall accuracy (95% CI) of multispectral MRI was 87% (80% to 94%), sensitivity was 85% (78% to 92%), and specificity was 92% (86% to 98%). There was good agreement between MRI and histological findings, with a value of $\kappa = 0.69$ (0.53 to 0.85).

Conclusions—Multispectral MRI can identify the lipid-rich necrotic core in human carotid atherosclerosis in vivo with high sensitivity and specificity. This MRI technique provides a noninvasive tool to study the pathogenesis and natural history of carotid atherosclerosis. Furthermore, it will permit a direct assessment of the effect of pharmacological therapy, such as aggressive lipid lowering, on plaque lipid composition. (Circulation. 2001;104:2051-2056.)

Key Words: magnetic resonance imaging n lipids n atherosclerosis n carotid arteries

There has been considerable interest in the past several years in characterizing “vulnerable plaques”: lesions of atherosclerosis thought to be associated with a higher risk for thromboembolic complications. More recently, however, there has been controversy over the term “vulnerable,” because it suggests the ability to predict future adverse events. Much of what is known about lesion characteristics associated with acute thromboembolic events is based on specimens harvested at the time of surgery or during post-mortem examination. This provides detailed information of lesion characteristics from a single time point but makes it difficult to determine which plaque features will result in future ischemic complications.

To determine which plaque features pose a higher risk for future ischemic events, we need an imaging tool that can identify presumptively high-risk plaque features in a nondestructive fashion, then prospectively follow up the patient until a clinical end point such as a stroke, transient ischemic attack, or acute coronary ischemic event occurs. Such a tool would permit serial, direct assessment of the effect of pharmacological interventions, such as aggressive lipid lowering, on atherosclerotic lesion size and lipid content.

High-resolution MRI is ideal for serial examination of the diseased arterial wall, because it is noninvasive and has superior capability for discriminating tissue characteristics compared with other imaging modalities.1–4 Soila et al5 and Maynor et al3 published early reports demonstrating that lipid components of atherosclerotic plaque can be distinguished with MRI. Toussaint et al4 noted that calcification, fibrous intimal tissue, and hemorrhage could be identified on the basis of T2 measurements of carotid plaques in vivo in a small series. Shinnar and colleagues6 subsequently demonstrated that MRI is capable of identifying carotid plaque constituents with high sensitivity and specificity ex vivo.
Recently, we reported findings on identifying fibrous cap characteristics and noted a high level of agreement between in vivo MRI and gross and histological examination of carotid endarterectomy specimens.7

Because histopathological studies8–12 suggest that the lesions at risk for plaque rupture are typically soft and contain a large necrotic core or intraplaque hemorrhage (IH), noninvasive detection of these morphological features could identify the “at-risk” plaque before the development of clinical symptoms. In this study, we report the overall accuracy of MRI for identifying the lipid-rich necrotic core (LR-NC) and recent IH in vivo in a larger series than previously reported. Furthermore, we used multispectral MR techniques (T1-weighted [T1W], T2-weighted [T2W], proton density–weighted [PDW], and 3D-time-of-flight [TOF]), because it has been suggested that information from multiple contrast-weighted images may be superior to single contrast-weighted images for identifying lesion components.6,13

Methods

Study Population

Between September 1997 and March 1999, 18 consecutive patients scheduled for carotid endarterectomy at the University of Washington Medical Center or VA Puget Sound Health Care System were recruited for the study after having given informed consent. Institutional review boards of each facility approved the consent forms and study protocols. All patients underwent a carotid artery MRI examination within 1 week of the surgical procedure to reduce potential errors in the correlation between image and pathology.14

MRI Protocol

Patients were imaged with a custom-designed phased-array surface coil in a 1.5-T GE Signa Scanner (Horizon EchoSpeed, version 5.8, GE Medical Systems). A standardized protocol was used to obtain 4 different contrast-weighted images (TOF, T1W, PDW, and T2W)15 of the carotid arteries 2 cm proximal and 2 cm distal to the bifurcation. Fat suppression was used to reduce signal from subcutaneous tissues, and a zero-filled Fourier transform was used to reduce pixel size and minimize partial-volume artifacts. Parameters for the 3 sequences were (1) double inversion recovery T1W 2D fast spin-echo (FSE) (TR/TE 800/9.3 ms, field of view [FOV] 13 cm, thickness 2 mm, 256×256 matrix, number of excitations [NEX] 2); (2) cardiac-gated, shared echo FSE for PDW and T2W images (TR 3RR, first echo TE 20 ms, second echo TE 40 ms, FOV 13 cm, thickness 2 mm, 256×256 matrix, NEX 2); and (3) 3D-TOF (TR/TE 23/3.8 ms, flip angle 25°, FOV 13 cm, thickness 2 mm, 256×256 matrix, NEX 2). Best voxel size was 0.254×0.254×2.0 mm.

Slice levels were centered at the carotid bifurcation of the operative side in each patient. This protocol generated 4 to 6 image locations per patient examination that could be compared with histological sections of the endarterectomy specimens. The 18 patient examinations provided 101 prescribed locations, with 4 matched carotid images of different contrast weightings (3D-TOF, T1W, PDW, and T2W) at each location. Image quality was rated on a 5-point scale dependent on the overall signal-to-noise ratio (SNR): grade 1, low SNR limits use, arterial wall and vessel margins are unidentifiable; grade 2, marginal SNR, arterial wall is visible, but the substructure, lumen, and outer boundaries are indistinct; grade 3, marginal SNR, wall structures are identifiable, but lumen and outer boundaries are partially obscured; grade 4, high SNR with minimal artifacts, vessel wall, lumen, and adventitial margins are well defined; and grade 5, high SNR without artifacts, wall architecture depicted in detail, lumen and adventitial boundary are clearly defined. Images from a location were excluded from the study if ≥2 of the images had an image-quality grade ≤2.

TABLE 1. MRI Criteria Used to Identify Plaque Tissue Components

<table>
<thead>
<tr>
<th>Plaque Component</th>
<th>MR Contrast Weighting</th>
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<tbody>
<tr>
<td></td>
<td>TOF</td>
</tr>
<tr>
<td>Recent IH</td>
<td>+</td>
</tr>
<tr>
<td>LR-NC</td>
<td>+/−</td>
</tr>
<tr>
<td>Intimal calcifications</td>
<td>−</td>
</tr>
</tbody>
</table>

Interpretation of signal intensity was made with reference to the immediately adjacent SCM muscle. Some plaque components had variable signal intensity on TOF and T2W images, which may be secondary to the presence/admixture of other tissue types in the same region. + indicates hyperintense compared with reference tissue, +/− indicates signal intensity similar to reference, and − indicates hypointense to reference tissue.

Image Review

To facilitate spatial correlation with the histological slides, carotid images were divided into quadrants. The primary axis was defined by a line through the lumen centers of the internal and external carotid arteries in the cross section, cephalad of the common carotid artery bifurcation. A second axis, perpendicular to this axis, was made through the center of the internal carotid artery. The orientation of the primary axis was maintained and used to define the axes in the common carotid artery.

The MR images from each of the contrast weightings were examined by 2 readers (C.Y. and L.M.M.). The readers reviewed the images together, and a consensus decision was made regarding the presence or absence of an LR-NC or IH for each location. The particular vessel quadrant and distance from the bifurcation of each region of interest were recorded. Interpretation of signal intensity was made with reference to the immediately adjacent sternocleidomastoid (SCM) muscle, a well-described methodology.2,16

To establish criteria for identifying LR-NC and acute IH, a pilot study was first performed on a separate data set to determine the signal characteristics of these tissue types under different contrast weightings. The findings of that study are summarized in Table 1. The most informative type was found to be T1W images, in which LR-NC appeared hyperintense relative to the SCM muscle and IH appeared isointense to hyperintense. The best discriminator between LR-NC and IH was then found to be TOF images, in which IH regions appeared hyperintense and LR-NC appeared isointense. Finally, both tissue types showed considerable variation in relative intensity on T2W and PDW images. Often the LR-NC and IH regions appeared hypointense on T2W and PDW images, as previously reported,2,16 but in many cases, these regions were isointense.

On the basis of the pilot study findings, a region of interest was adopted for identifying LR-NC and IH. First, the T1W images were examined to select regions that were hyperintense or isointense compared with the SCM muscle. Then, each of these regions was examined on the corresponding TOF image. If the region was hyperintense on the TOF image, it was categorized as IH; if it was isointense on the TOF image and hyperintense on the T1W image, it was categorized as LR-NC. Finally, if the T1W (or TOF) image had poor image quality, the T2W and PDW images were consulted, and a region was categorized as LR-NC or IH if it was hypointense after calcium had been ruled out.

Histological Processing

After carotid endarterectomy, the specimens were fixed in formalin, decalcified, and embedded in paraffin. Sections were sectioned (10 μm thick) every 0.5 to 1.0 mm throughout the length of the endarterectomy specimen and stained (hematoxylin-eosin, Mallory’s trichrome). The slides were independently evaluated by a reviewer (M.S.F.) who was unaware of the imaging results and were categorized according to the histopathological classification described by Stary et al.17 from the Committee on Vascular Lesions of the Council of Atherosclerosis, American Heart Association. Regions containing an LR-NC were distinguished from areas of dense, collagen-rich
fibrous intimal tissue and regions with loose connective tissue matrix by criteria similar to those described by Moreno et al. Acute IH was identified by the presence of relatively intact red blood cells with a polymorphonuclear infiltrate.

Correlation Between MRI and Histology
After both MR images and histological sections were reviewed and categorized, comparison was performed. Given the difference in slice thickness between MRI (2 mm) and histological cross sections (10 μm, every 0.5 to 1.0 mm), 3 to 4 histological sections for each MR image location were selected on the basis of the relative distance of the MRI and histological section from the common carotid bifurcation. To correct for shrinkage of the endarterectomy specimen during histological processing, additional measures were used for matching the MRI and histological sections. First, the gross morphological features of the lumen and vessel wall, such as the overall shape of the lumen and wall, were compared. Second, the location of large calcified regions, which appear hypointense on MRI, aided in matching the cross sections at each location. An agreement between MRI and histology was defined as the presence of an LR-NC or IH region in the same quadrant on the MRI section and in all 3 to 4 of the matched histological sections.

Data Analysis
All calculations were made with SPSS for Windows (version 7.5.1). In addition to sensitivity and specificity, Cohen’s κ with 95% CI was computed to quantify the agreement between the MRI findings and histology. A value of κ>0.7 was used to indicate a high level of agreement.

Because multiple image locations from each patient would be used for the statistical evaluation, the interdependence of each location for a given patient examination was assessed by use of a κ statistic. A value of κ<0.4 indicates weak or no interdependence.

Results
Of the 101 image locations obtained, 8 were excluded because of poor image quality (rating ≤2), typically related to patient motion or deep location of the vessels in the soft tissues of the neck. Three locations could not be used because of distortion of the specimen by histological processing. Therefore, 90 carotid locations were available for comparison.

Figure 1 presents the typical appearance of an LR-NC region in images with the 4 contrast weightings. Figure 2 shows an area of acute IH characterized by hyperintense signal.

Sensitivity and Specificity Evaluation
Examination of the 4 matched carotid images (TOF, T1W, PDW, and T2W) for each of the 90 carotid locations demonstrated 58 LR-NC/IH regions, and all but 2 were
confirmed histologically (Table 2). Overall accuracy (95% CI) was 87% (80% to 94%), sensitivity was 85% (78% to 92%), specificity was 92% (86% to 98%), and \( \kappa = 0.69 \) (0.53 to 0.85). The sensitivity and specificity for identifying LR-NC regions without IH were 98% (96% to 100%) and 100% (92% to 100%), respectively. The accuracy of MRI for identifying acute IH alone could not be estimated with confidence, because only 16 cases of isolated acute IH occurred in this series. Cross-tabulation for the interdependence of pertinent regions in the same patient showed a value of \( \kappa = 0.48 \), indicating a low-to-moderate correlation among the regions identified. The impact of this low-to-moderate correlation results in slightly wider confidence levels than those noted.

### Discussion

Advances in our understanding of vascular biology and the promise of new therapies emphasize the need to develop a means to reliably identify and monitor the development of the high-risk vulnerable atherosclerotic plaque. For this objective, MRI has several advantages. It is a noninvasive modality that can potentially characterize tissue at the molecular level.\(^4\) MR images can be analyzed quantitatively. Excellent reproducibility of location can result in accurate serial studies. Currently, MR is being used to characterize human lesions of atherosclerosis\(^23\)–\(^26\) and has high diagnostic accuracy for identifying carotid plaque constituents\(^2\)\(^5\)–\(^6\). This study demonstrates the capabilities of in vivo multispectral MR analysis in the characterization of advanced lesions of atherosclerosis. Although the scope of this study was limited to an assessment of the LR-NC and acute IH, its clinical value is apparent. Numerous histopathological studies suggest an important role for soft plaque features, such as an LR-NC and acute IH, in the pathogenesis of plaque rupture.\(^25\)–\(^27\) Furthermore, noninvasive identification of the lipid core may have important application in lipid-lowering clinical trials.

The availability of multispectral MR, especially TOF-based bright-blood and spin-echo–based black-blood techniques, was an important factor in the high sensitivity and specificity achieved. As demonstrated in Figures 1 and 2, no single contrast weighting was used to identify the LR-NC and IH regions accurately. Rather, a combination of reviewing images with different contrast weightings provided the most comprehensive evaluation. Figures 3 and 4 illustrate the importance of multiple contrast weightings to identify other structures of interest in advanced atherosclerotic plaques.

Previous studies have demonstrated good sensitivity and specificity for identifying plaque constituents by use of spin-echo–based sequences (primarily T2W and PDW imaging) on ex vivo MRI.\(^2\)\(^6\) This study indicates that TOF and T1W images are also valuable for identifying plaque constituents and may be the preferred imaging methods for identifying LR-NC and IH in vivo. Specifically, we found that both acute IH and LR-NC regions can appear hyperintense on T1W imaging compared with the adjacent SCM muscle. Furthermore, acute IH can be distinguished from the LR-NC by the presence of high signal intensity in the TOF images. This finding is consistent with those reported by Moody et al\(^28\) on the identification of acute deep venous thrombosis and further supports the argument that gradient echo–based TOF images should be optimized for atherosclerotic plaque characterization.\(^7\)

Our reliance primarily on T1W and TOF images to identify LR-NC and IH is somewhat at odds with previously reported

### Table 2. Test Performance of Multispectral MRI for Identifying Regions of LR-NC and Acute IH

| MRI + | Histology + | 56 | 2 |
| MRI − | Histology − | 10 | 22 |

Accuracy (95% CI) = 87% (80% to 94%), sensitivity = 85% (78% to 92%), specificity = 92% (86% to 98%), and \( \kappa = 0.69 \) (0.53 to 0.85).

### Table 3. Test Performance of Multispectral MRI for Identifying Regions of LR-NC Without Acute IH

| MRI + | Histology + | 44 | 0 |
| MRI − | Histology − | 1 | 39 |

Sensitivity = 98% (96% to 100%), specificity = 100% (92% to 100%), and \( \kappa = 0.98 \) (0.93 to 1.0).

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**Figure 3.** Importance of multiple contrast-weighted image examination. TOF image reveals distinct, irregular lumen boundary that is not well defined by T1W, PDW, and T2W images. Histological examination demonstrated that material intruded into lumen contained calcified nodules.
techniques for identifying these tissues. Although we found that many of the necrotic core regions appeared hypointense on T2W imaging, consistent with previous reports in the literature, \textsuperscript{2,6} we also found that the LR-NC in other regions was isointense on T2W images. One explanation for the discrepancy is that the population of patients and complexity of lesions examined differs from those studied in previous reports. In this study, we examined cross sections from the region of the proximal common carotid artery, carotid bifurcation, and distal internal carotid artery. The lesions identified at the carotid bifurcation typically demonstrated atheroma in its advanced stages. On histological examination, the necrotic cores at this location had a complex composition demonstrating the presence of crystalline cholesterol (cholesterol clefts), which would be expected to appear hypointense on T2W imaging. Some of the cores at the bifurcation, however, also contained variable amounts of necrotic debris, proteinaceous material, and IH of various ages that could affect signal intensity on the PDW and T2W images of these regions (old lesions). \textsuperscript{29} Conversely, lesions in the proximal common carotid artery tended to be less complex and demonstrated features characteristic of earlier atherosclerosis, with less heterogeneity of tissue types within the plaque core (new lesions). LR-NCs in the proximal common carotid artery typically appeared hypointense on T2W imaging, consistent with previous reports in the literature.

The variation in signal intensities on PDW and T2W images most likely reflects the integrity of red blood cells and state of hemoglobin within the region. Both of these factors strongly affect tissue T2 relaxation times but have less effect on T1W/TOF images. This suggests that PDW and T2W images may play a vital role in subcategorizing necrotic tissue types by age or other characteristics.

Future Work
Ultimately, our aim is to develop automated analysis techniques for identifying and quantifying atherosclerotic tissue types. This study, which uses qualitative image interpretation by expert readers, provides the necessary preliminary groundwork. Subsequent development of quantitative image analysis techniques, such as identifying tissue boundaries and automatically classifying tissue types, present significant challenges. Signal variations introduced by surface coils hinder our ability to establish brightness thresholds for different tissue types. Patient motion leads to misregistration of images taken at different times, which makes the integration of multispectral information difficult. Although challenging, development of robust quantitative analysis tools is essential for future multicenter, longitudinal studies to reduce interpreter variability. We are therefore developing image processing techniques to address some of the issues related to quantitative analysis. \textsuperscript{30,31}

Conclusions
The results of this study demonstrate that multispectral MR imaging can identify LR-NCs and IH in advanced atherosclerotic carotid plaques with high sensitivity and specificity. The ability to identify these high-risk plaque constituents noninvasively will be valuable in prospective, longitudinal studies examining the pathogenesis of the vulnerable plaque. Furthermore, identification of the lipid-rich core with MRI has significant potential application in lipid-lowering clinical trials.

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References


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