In Vivo Quantitative Measurement of Intact Fibrous Cap and Lipid-Rich Necrotic Core Size in Atherosclerotic Carotid Plaque
Comparison of High-Resolution, Contrast-Enhanced Magnetic Resonance Imaging and Histology

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Background—Previous studies with contrast-enhanced magnetic resonance imaging (CEMRI) have shown that the fibrous cap (FC) in atherosclerotic carotid plaques enhances with gadolinium-based contrast agents. Conversely, the lipid-rich necrotic core (LR-NC), lacking both vasculature and matrix, shows no or only slight enhancement. The goal of this study was to assess whether CEMRI can be used to accurately measure the dimensions of the intact FC and LR-NC.

Methods and Results—Twenty-one patients scheduled for carotid endarterectomy were imaged with a 1.5-T scanner. Precontrast images and CEMRI were obtained. One hundred eight locations with an intact FC were matched between MRI and the excised histology specimens. Quantitative measurements of FC length along the lumen circumference, FC area, and LR-NC area were collected from CEMRI images and histology sections. Blinded comparison of corresponding MR images and histology slices showed moderate to good correlation for length (r=0.73, P<0.001) and area (r=0.80, P<0.001) of the intact FC. The mean percentage LR-NC areas (LR-NC area/wall area) measured by CEMRI and histology were 30.1% and 32.7%, respectively, and were strongly correlated across locations (r=0.87, P<0.001).

Conclusions—In vivo high-resolution CEMRI is capable of quantitatively measuring the dimensions of the intact FC and LR-NC. These new parameters may be useful to evaluate plaque vulnerability and provide continuous variables for characterizing the intact FC and LR-NC in progression and regression studies. (Circulation. 2005;112:3437-3444.)

Key Words: magnetic resonance imaging ▪ carotid arteries ▪ contrast media ▪ atherosclerosis ▪ lipids
Previous studies by Yuan et al\textsuperscript{10} and Wasserman et al,\textsuperscript{11} using gadolinium-based, contrast-enhanced MRI (CEMRI), have shown that postcontrast T1-weighted (T1W) MR images helped discriminate the FC from the necrotic core. Wasserman et al\textsuperscript{11} showed that the contrast-to-noise ratio was as good as or better than that with T2W MR images but with approximately twice the signal-to-noise ratio (postcontrast images, 36.6 \pm 3.6; T2W images, 17.5 \pm 2.1; \textit{P}<0.001). Yuan et al\textsuperscript{10} showed that the enhancement of fibrous tissue was moderate to strong (79.5\% \pm 29.1\%), whereas the LR-NC enhanced only slightly (28.8\% \pm 20.1\%). The differences between enhancement of fibrous tissue and LR-NC were significant.

This study aimed to (1) test the hypothesis that CEMRI, by facilitating discrimination of the enhancing FC and the underlying LR-NC, can provide accurate quantitative measurements of the intact FC and LR-NC in advanced carotid atherosclerotic plaques in vivo, including those with intraplaque hemorrhage and calcification, and (2) compare the differences between FC area measured on T2W images versus those measured by CEMRI.

### Methods

#### Study Population

Between March 2001 and May 2003, 30 consecutive patients scheduled for carotid endarterectomy at either the University of Washington Medical Center or VA Puget Sound Health Care System were recruited for this study. Institutional review boards at each facility approved the consent forms and study protocols. All subjects underwent a carotid artery MRI examination within 1 week before their surgery. All subjects were either symptomatic patients who had experienced a transient ischemic attack or stroke within 6 months of their surgery and had >50\% carotid stenosis or were asymptomatic with >80\% carotid stenosis.

#### MRI Protocol

Patients were imaged with a 1.5-T MR scanner (Signa Horizon EchoSpeed, General Electric Health Care) and phased-array surface coils (Pathway MRI Inc). Precontrast MR images that included double-inversion-recovery T1W, proton density–weighted (PDW), T2W, TOF, and postcontrast double-inversion-recovery T1W MR images of carotid arteries were obtained with a previously published standardized protocol (for double-inversion-recovery T1W, repetition time/echo time/inversion time, 800/10/650 ms; for cardiac-gated PDW and T2W, repetition time/echo time, 3RR, 20/40 ms; for TOF, repetition time/echo time, 23/3.8 ms).\textsuperscript{10} A gadolinium-based contrast agent (Omniscan, Amersham Health), 0.1 mmol/kg (0.2 ml/kg) body weight, was injected intravenously with a power injector, and acquisition of postcontrast T1W images occurred 6 to 10 minutes after injection. The total protocol time for each patient was \textasciitilde 50 minutes. All images were obtained with the following parameters: field-of-view of 13 cm, matrix size of 256 \times 256, slice thickness of 2 mm, 2 signal averages, and longitudinal coverage of 20 to 24 mm (10 to 12 locations). A zero-filled Fourier transform was used to reduce pixel size (to 0.25 \times 0.25 mm\textsuperscript{2}), preserve inherent image resolution (0.5 mm), and minimize partial-volume artifacts due to image zooming and display.

#### Histology Sample Processing and Criteria

Carotid endarterectomy specimens were excised intact without disruption of the luminal surface of the plaque. The specimen was fixed in 10\% neutral buffered formalin, decalcified in 10\% formic acid, and embedded en bloc in paraffin. Sections (10 \mu m thick) were taken every 1.0 mm in the common carotid artery and every 0.5 mm in the internal carotid artery throughout the length of the specimen and stained with hematoxylin-eosin and Mallory’s trichrome stains.

Histological classification of the specimens was performed according to criteria established by the American Heart Association Committee on Vascular Lesions.\textsuperscript{6,7} All histology sections were examined by 1 reviewer (M.S.F.), who was unaware of the MRI data. Locations with an intact FC and underlying LR-NC were selected and then matched to MRI images.

Histology sections corresponding to 218 image locations were initially reviewed for the thickness of the LR-NC. Because the focus of the study was to measure the intact FC and necrotic core, 110 locations were excluded because of FC rupture (n=18), early lesions without necrotic cores or FCs (n=90), or poor image quality (n=2), leaving 108 locations for analysis. FC components such as dense collagen matrix, loose (proteoglycan) matrix (LM), calcification, hemorrhage, neovascularure, and inflammatory cell infiltrate were recorded.

#### Matching of MRI and Histology Images

Matching of MR to histology findings used precontrast T1W and TOF images. Histology sections were matched to images based on the relative distance from the common carotid bifurcation, the gross morphological features such as lumen and wall size and shape, and the presence/absence of large regions of dense calcification, which can be readily identified by MRI and histology.\textsuperscript{22} In matching, use of multiple internal landmarks is necessary to accommodate shrinkage of the specimens during fixation and processing. Shrinkage may average 30\% in the length and 15\% in the width, and it varies by plaque composition.\textsuperscript{23} The use of precontrast T1W and TOF images for matching kept the histologist blinded to the T2W and CEMRI results. To further ensure blinding, a 1-month interval was used between matching and subsequent evaluation of the FC and LR-NC.

#### Image Review

An image-quality rating (5-point scale: 1=poor, 5=excellent) for each contrast weighting was assigned to all MR images\textsuperscript{12} before reviewing imaging locations with an image quality \textasciitilde 2 were excluded from the study. Each imaging location contained 4 precontrast (T1W, PDW, T2W, and TOF) images and 1 postcontrast (T1W) image.

#### Measurement of the LR-NC and FC

The quantitative measurements of LR-NC area and the dimensions of the FC (area and length along the lumen circumference) were collected from postcontrast T1W images (Figure 1), by using a custom image analysis tool (QVAS)\textsuperscript{13} with an image magnification of 4000\%. QVAS permits simultaneous display of up to 6 contrast weightings and provides automated and manual tools for boundary identification, registration, and segmentation. On completion of image review, QVAS produces a comprehensive lesion report that summarizes the areas, volumes, and thicknesses of the plaques and plaque components.

With the noncontrast T1W image as a baseline for comparison, the LR-NC area was identified on the postcontrast T1W image as the area with no or slight contrast enhancement compared with the surrounding, more strongly enhanced fibrous tissues. Regions of dense calcification might also demonstrate little enhancement, but these are characterized by defined areas with hypointense signals (relative to the signal of the adjacent sternocleidomastoid muscle) on all precontrast weightings,\textsuperscript{14,15} leaving 108 locations for collaborative work with the MRI team. To account for specimen shrinkage, the LR-NC was also calculated as a percentage of the total plaque area, defined as the region between the lumen boundary and outer wall boundary on both precontrast T1W MRI and histology images.

Using the adjacent LR-NC as a reference, we defined the enhancement of the FC on postcontrast T1W images as “strong enhancement” if the FC appeared more than twice as bright than the LR-NC and “moderate enhancement” if the FC appeared only slightly brighter than the LR-NC. The FC area was measured by identifying the region with moderate to strong enhancement between the dark lumen and the LR-NC. The length of the FC was measured along the lumen surface between 2 lines drawn from the margins of the LR-NC, perpendicular to the lumen surface (Figure 1).

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\textsuperscript{1} Yuan et al,\textsuperscript{10} Wasserman et al,\textsuperscript{11} QVAS 5-point scale: 1=poor, 5=excellent; PDW, proton density–weighted; TOF, time-of-flight; QVAS, quick voxel analysis system.
Comparison of FC Area Measured by T2W Images and by CEMRI

The FC area was also measured on the basis of T2W images alone. On T2W images, the FC appeared hyperintense relative to the adjacent lumen and underlying LR-NC. Quantitative comparisons with histology and CEMRI were made.

Measurement Reproducibility

All MR images were examined by 1 radiologist (J.C.) who was blinded to the histology findings. However, to assess intraobserver and interobserver reproducibility, MR images of 12 randomly selected subjects (with 69 locations) were reevaluated by 2 reviewers (J.C. and N.T.) 8 months after the initial review.

Data Analysis

The method of Bland and Altman was used to assess the agreement between measurements from MRI and histology. With the histology result as the reference measurement, the Bland-Altman method provides estimates of bias and precision and a plot that is useful for determining whether bias and precision are constant across the range of a measurement. To accommodate the shrinkage that occurs in carotid plaque specimens during histological processing, agreement between MRI and histology measurements was also assessed with the Pearson correlation coefficient ($r$), with the location as the unit of observation. Because of potential statistical dependence of measurements obtained from multiple locations within a patient, all probability values for any null hypothesis of zero correlation were calculated according to the nonparametric bootstrap method (with n=999 resamples of the patient as the unit of sampling). Although a previous study of some types of MRI plaque measurements has shown that they are relatively independent among locations in the same artery, it is appropriate to protect against potential dependence. With the bootstrap method, the significance level $P$ for a correlation coefficient was calculated by noting the largest (1 − $P$) confidence level of any 2-sided confidence interval (CI) not including the null value of $r$=0. The CI was constructed by using the percentile method with an empirical bootstrap distribution. The statistical significance of the difference between 2 correlation coefficients (correlation of CEMRI area of FC with T2W area and correlation of histology FC area with T2W area) was also calculated by using the bootstrap. Means were compared between 2 different categories of locations with an intercept-only linear-regression model and generalized estimating equations. The bias and precision of MRI measurements relative to histology measurements are presented as the mean difference and the SD of the difference of the 2 measurements. To determine intraobserver and interobserver reproducibility measurements, intraclass correlation coefficients (ICCs) with 95% CIs were calculated to measure the level of agreement. The CIs for ICC were calculated with the bootstrap method. Cohen’s $\kappa$ was calculated for the agreement of 2 independent determinations of FC enhancement by CEMRI, with the CI determined from the bootstrap method. The independent-samples $t$ test and Fisher’s exact test were used to compare continuous and categorical characteristics, respectively, between patients included in and excluded from the present study. All analyses were conducted with SPSS 12.0 (SPSS, Inc) and R 2.0.0 (R Foundation for Statistical Computing) software.

Results

Of the 30 original patients, 9 were excluded from the study as a result of image quality ≤2 secondary to patient motion (6 patients); marked angulation and tortuosity of the proximal internal carotid artery, resulting in partial-volume averaging artifacts (2 patients); and damage of the endarterectomy specimen precluding histological analysis (1 patient). The mean characteristics of the 21 patients used in data analysis are described in Table 1. All of the mean clinical parameters described in Table 1 were very similar between the included and excluded patients, and none differed significantly. Imaging results from 1 of the 21 remaining patients are shown in Figure 1 with its corresponding histology results. On histological examination, intraplaque hemorrhage was detected in...
47 of 108 (43.5%) locations, and calcification was identified in 58 (53.7%) locations.

**Comparison of FC Measurements by Histology With Those by CEMRI**

The matched CEMRI and histology slices showed moderate to good correlation for length \( r = 0.73, P < 0.001 \) and area \( r = 0.80, P < 0.001 \) of an intact FC (Table 2). Comparison plots of the FC measurements between MRI and histology as well as Bland-Altman plots are shown in Figure 2.

**FC Enhancement**

Of the 108 locations, 84 (77.8%) showed strong enhancement and 24 (22%) showed moderate enhancement (Figures 1, 3, and 4). Substantial differences in the composition of the FCs were noted on histological examination. Sixty (56%) of the FCs were predominantly collagen rich, whereas 48 (44%) of the caps were composed of LM. All 24 of the locations exhibiting moderate enhancement were collagen rich. The remaining 36 collagen-rich FCs that demonstrated strong enhancement were found by histology to contain neovascu- lature or inflammatory cell infiltrates within the FC. All of the 48 LM-predominant FCs exhibited strong enhancement on the postcontrast T1W images. The extent of enhancement was rated twice on the same images, separated by a 10-month interval (each time as “strong” or “moderate”). The rating of the 2 assessments was very consistent, with a 96.3% agreement and Cohen’s \( \kappa = 0.89 \) (95% CI, 0.77 to 0.98).

**Comparison of LR-NC Measurements by**

**Histology With Those by CEMRI**

The LR-NC areas measured by MRI and histology were 16.49 ± 10.28 mm\(^2\) (mean ± SD) and 12.42 ± 8.25 mm\(^2\), respectively, and were strongly correlated \( r = 0.84, P < 0.001; \) Table 2. The comparison and Bland-Altman plots of the LR-NC between MR and histology are shown in Figure 5. There was a trend toward increasing bias with increasing magnitude of LR-NC area, as measured by CEMRI, relative to the area as measured by histology (Figure 5C). However, this trend in bias disappeared when the LR-NC area was expressed as a percentage of wall area (Figure 5D). The mean percentages of LR-NC areas were 30.1 ± 12.5% and 32.7 ± 12.3% for MRI and histology, respectively, and were strongly correlated across locations \( r = 0.87, P < 0.001 \) (Figure 5).

**Reproducibility**

Reproducibility was determined from repeated, independent readings of measurements of 69 locations from 12 patients. Intraobserver reproducibility was excellent for LR-NC area (ICC, 0.87; 95% CI, 0.70 to 0.92), FC area (ICC, 0.72; 95% CI, 0.57 to 0.84), and length of FC (ICC, 0.80; 95% CI, 0.70 to 0.85). Interobserver reproducibility was excellent for LR-NC area (ICC, 0.89; 95% CI, 0.81 to 0.93), FC area (ICC, 0.78; 95% CI, 0.68 to 0.86), and length of FC (ICC, 0.81; 95% CI, 0.69 to 0.88).

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**TABLE 2. Comparison Between Histology and CEMRI Measurements of the FC and LR-NC**

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Mean ± SD MRI</th>
<th>Mean ± SD Histology</th>
<th>Difference, CEMRI—Histology Mean (Bias)</th>
<th>SD (Precision)</th>
<th>Pearson Correlation</th>
<th>( P^{*} ) (Pearson Correlation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FC Length, mm</td>
<td>5.08 ± 2.36</td>
<td>4.44 ± 2.17</td>
<td>0.64</td>
<td>1.68</td>
<td>0.73</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FC Area, mm(^2)</td>
<td>5.58 ± 2.71</td>
<td>4.13 ± 2.45</td>
<td>1.45</td>
<td>1.68</td>
<td>0.80</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LR-NC Area, mm(^2)</td>
<td>16.49 ± 10.28</td>
<td>12.42 ± 8.25</td>
<td>4.07</td>
<td>5.55</td>
<td>0.84</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Based on the bootstrap method (see text for details).
Discussion

Findings from this study demonstrate that gadolinium CEMRI not only permits the identification of the intact FC, as suggested by previous studies, but, more important, also allows accurate morphological measurements of the FC. In vivo quantification of FC length and area by CEMRI was correlated well with histology measurements (the "gold standard") from intact carotid endarterectomy specimens (r = 0.73 to 0.80, P < 0.001). Furthermore, results from this study suggest that the addition of gadolinium-based contrast enhancement improves the performance of MRI for measuring the size of the LR-NC. Recently, in a study from our group, Saam et al reported a good correlation for LR-NC measurement between non-CE multisequence MRI and histology (r = 0.75) in a similar population of patients (there was an 8-patient overlap between these 2 studies). This study differs from previous studies by providing a quantitative measurement of the intact FC and LR-NC by CEMRI. The novelty of this study is that it is the first to test whether CEMRI is capable of quantifying intact FC and LR-NC size in atherosclerotic carotid plaque.

In comparison, measurements of LR-NC area by CEMRI were more strongly correlated with histology (r = 0.84). After normalizing the data to adjust for shrinkage of histological specimens, the average percentage LR-NC area (LR-NC area/wall area) was 30.1% and 32.7% for MRI and histology, respectively (r = 0.87). These findings indicate that gadolinium-based contrast enhancement further extends the potential of MRI in tracking the natural progression of disease, as well as the effect of therapy for the regression of carotid atherosclerosis.

FC Enhancement

The underlying mechanism of FC enhancement is likely to be multifactorial. Wasserman et al noted that gadolinium-based contrast agents are known to distribute into the extracellular fluid space and cause a greater degree of enhancement in the vessel wall and FC, which may be due to (1) increased washin of gadolinium-based contrast agent (increased permeability); (2) increased volume of distribution (increased extracellular volume); or (3) decreased washout. In this study, the degree of enhancement of the FC varied depending on its composition. FCs that were predominantly composed of organized, dense, collagen demonstrated moderate enhancement on the postcontrast T1W images. FCs with LM, neovasculature, and inflammatory cell infiltrates were associated with stronger enhancement on CEMRI, consistent with the notion that greater enhancement is seen with increased permeability and increased volume of distribution within the cap.
The potential to quantify the dimensions of the FC and determine its composition in a serial, noninvasive fashion has significant implications. Such a tool would permit prospective, serial studies to test the hypothesis that FC atrophy and caps with inflammatory cell infiltrates are at the highest risk for cap rupture, plaque volume progression, transient ischemic attack, and stroke. To fully achieve this potential, objective, quantitative methods to differentiate the moderate enhancement of collagen-rich FCs from the strong enhancement of caps with neovasculature, LM, and inflammatory cell infiltrate are needed. Using a novel, dynamic spoiled gradient-echo CEMRI acquisition protocol to measure the transfer constant of blood plasma and the extracellular space, Kerwin et al. demonstrated a strong correlation between dynamic CEMRI findings and the amount of overall plaque neovasculature and macrophage infiltrate seen on histology. However, because of lower resolution of the dynamic spoiled gradient-echo CEMRI technique, quantitative measures of neovasculature and inflammatory cell infiltrate localized to the small region of the FC are currently not robust; however, improvements in hardware (higher-field scanners, coil design) and image acquisition protocols will make this feasible in the near future.

Comparison of T2W and CEMRI

Previous studies have examined the ability of T2W imaging to measure the FC and LR-NC. In a study of ex vivo T2W
MR images that investigated FC thickness and lipid core volume, Serfaty et al.\textsuperscript{16} found the lipid core to be overestimated, therefore exaggerating the vulnerability of numerous plaques. In an in vivo study of carotid plaque, Trivedi et al.\textsuperscript{9,17} also used T2W MRI and reported that the thickness and area ratio of the FC and lipid core of carotid atherosclerotic plaques showed good to strong agreement with histology. However, lesions with intraplaque hemorrhage and calcification were excluded from the study, thereby limiting its application to a subset of patients with atherosclerosis. Of note, in our study, intraplaque hemorrhage was identified in 47 of 108 locations (43.5%), and calcification was seen in 58 of 108 locations (53.7%) on histological examination.

Prior studies\textsuperscript{28,29} have shown that hemorrhage within the LR-NC may increase signal intensity on T2W images, depending on the age of the hemorrhage, thereby obscuring the boundary between the relatively hyperintense FC and the underlying LR-NC. In contrast, hemorrhage within the LR-NC is associated with only slight enhancement on CEMRI and is easily differentiated from surrounding enhanced fibrous tissue. Consistent with this observation, when we compared the difference between T2W and CEMRI measurements, the FC areas measured by T2W were substantially larger than those measured by CEMRI in plaques that had hemorrhage within the LR-NC on histology.

**Limitations**

Nine (30%) of 30 patients were excluded from final analysis in this study, with the majority of cases related to poor image quality secondary to patient motion. Contributing factors for motion artifact may include the longer scan duration required by addition of postcontrast imaging and patient anxiety about upcoming surgery, which was typically scheduled the day before the operation. Prospective, clinical trials or longitudinal, serial MRI studies in nonoperative patients should incorporate image-based exclusion criteria for enrollment, including depth of vessel, tortuosity, and excessive motion artifact. Continuing improvements in MRI hardware and acquisition protocols will improve image quality, reduce scan time, and reduce the number of excluded cases. Furthermore, higher resolution achieved through advances in technology, such as higher-field (3 T) MRI, is needed to improve precision and permit measurements of not only the FC area but also the thickness of the cap.

Pathological examination of the excised carotid endarterectomy specimens was used as the gold standard for assessing the CEMRI findings. However, histological processing results in
shrinkage of the specimen and makes comparison of absolute values difficult. In addition, some variability arises because of differences in slice thickness in the z direction on MRI (2 mm) and histology (10 μm).

Finally, studies designed to assess interscan reproducibility of CEMRI for measuring the FC and LR-NC are needed before this method can be used in prospective, clinical studies. Image analysis software is currently under development that will semiautomatically determine the percentage of enhancement and show a percentage change map. Such tools may improve overall accuracy and reproducibility in the evaluation of enhancement boundaries.

Conclusions
In vivo high-resolution CEMRI can provide quantitative measurements of the intact FC and LR-NC. This new capability may be useful in prospective studies evaluating plaque vulnerability by providing continuous variables for measuring the FC and LR-NC in progression and regression studies. CEMRI visualization and measurement of the FC may lead to new insights into the in vivo dynamics of plaque composition and vulnerability. Accurate measurement of the LR-NC provides another parameter with which to follow up pharmacological intervention, such as lipid-lowering treatment. Finally, these capabilities may assist in the development and assessment of new therapies for the treatment of carotid atherosclerosis, which in turn may have a major impact on the morbidity and mortality of stroke.

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