Differentiation of Intraplaque Versus Juxtaluminal Hemorrhage/Thrombus in Advanced Human Carotid Atherosclerotic Lesions by In Vivo Magnetic Resonance Imaging

A. Kampschulte, MD; M.S. Ferguson, MT(ASCP); W.S. Kerwin, PhD; Nayak L. Polissar, PhD; B. Chu, MD, PhD; T. Saam, MD; T.S. Hatsukami, MD; C. Yuan, PhD

Background—Intraplaque hemorrhage and juxtaluminal hemorrhage/thrombus may differ in cause and clinical implications. This study tested the hypothesis that MRI can distinguish between intraplaque hemorrhage and juxtaluminal hemorrhage/thrombus and investigated the association between hemorrhage and underlying lesion types.

Methods and Results—Twenty-six patients scheduled for carotid endarterectomy were imaged with a 1.5-T GE scanner by a multicontrast-weighted MRI technique. Hemorrhages were identified with previously established MRI criteria, and differentiations were made between intraplaque and juxtaluminal hemorrhage/thrombus. Corresponding histology was used to confirm the magnetic resonance findings. Tissues underlying areas of hemorrhage/thrombus were histologically categorized according to modified American Heart Association criteria. Of 190 matched sections, 140 contained areas of hemorrhage by histology, of which MRI correctly detected 134. The sensitivity and specificity for MRI to correctly identify cross sections that contained hemorrhage were 96% and 82%, respectively. Furthermore, MRI was able to distinguish juxtaluminal hemorrhage/thrombus from intraplaque hemorrhage with an accuracy of 96%. The distribution of lesion types underlying hemorrhages differed significantly ($P=0.004$). Intraplaque hemorrhage had an underlying lipid-rich type IV/V lesion in 55% of histological sections, whereas juxtaluminal hemorrhage/thrombus had an underlying calcified lesion type VII in 70% of sections.

Conclusions—In vivo high-resolution MRI can detect and differentiate intraplaque hemorrhage from juxtaluminal hemorrhage/thrombus with good accuracy. The association of hemorrhage and lesion types suggests potential differences in origin. Noninvasive MRI therefore provides a possible tool for prospectively studying differences in origin of plaque hemorrhage and the association of plaque progression and instability. (Circulation. 2004;110:3239-3244.)

Key Words: magnetic resonance imaging | atherosclerosis | carotid arteries | plaque | hemorrhage

Carotid atherosclerosis is of unquestioned importance in the pathogenesis of cerebral ischemia. Although advances in the understanding and treatment of these lesions have been made, thrombotic complications of atherosclerosis remain the leading cause of morbidity and mortality in Western society.¹ ² Vulnerable lesions are typically described as containing a large necrotic core or intraplaque hemorrhage (IH) that is separated from the lumen by an unstable fibrous cap.³ ⁵ The association between IH and clinical symptoms is well documented, but conflicting conclusions have been drawn. Several investigators have reported that IH appears to be more common in plaques of symptomatic patients than in plaques of asymptomatic patients.⁶ ⁸ Recent studies indicate that IH is equally present in both.⁹ ¹² More specifically, IH without surface rupture appears to have little association with patient symptoms.¹⁰ ¹² However, juxtaluminal hemorrhage/thrombus (JLH/T) indicates erosion, ulceration, or rupture that can cause further thrombotic incidents and may be clinically significant.

To prospectively investigate this issue, a method for in vivo visualization is needed. High-resolution MRI, as a noninvasive imaging tool, has proved to be a modality with excellent capability for discriminating tissues of the carotid plaque, including the status of the fibrous cap, lipid-rich necrotic core, calcium, and hemorrhage.¹³ ²¹ Moody et al showed by using a single T1-weighted sequence that MRI could differentiate between stable versus unstable plaque by detecting a high signal within the plaque, contributing to hemorrhage,²² and demonstrated that these lesions are more
common on the ipsilateral side of symptomatic patients. Chu et al showed that combined use of magnetic resonance images with multiple contrast weightings could further subdivide hemorrhage by age. These authors highlighted the value of an imaging-based method to study plaque hemorrhage but did not attempt to differentiate between the location of the hemorrhage and the status of the fibrous cap. IH and JLH/T may have different origins and clinical relevance.

There are 2 purposes for the present study: first, to assess the ability of MRI to differentiate IH and JLH/T, and second, to investigate the connection between plaque components and hemorrhage using the American Heart Association (AHA) lesion classification. Such a correlation between a particular lesion type and either IH or JLH/T may suggest etiological differences between the 2.

### Methods

#### Study Population

Twenty-six 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 they provided informed consent. Institutional review boards of each facility approved the consent forms and study protocols. Patients were imaged 1 week before surgery to reduce potential errors in the correlation between images and histology.

#### 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 obtained 4 different contrast-weighted images (time-of-flight [TOF], T1-, proton density- [PD], and T2-weighted) of the carotid arteries in 2-cm segments by a reviewer (M.S.F.) who was unaware of the imaging results. The resulting voxel size ranged from 0.25×0.25×2.0 to 0.31×0.31×2.0 mm³, depending on neck size.

#### MRI Image Review and Criteria

Image quality was rated per artery for each contrast weighting on a 5-point scale (1 = poor, 5 = excellent) dependent on the overall signal-to-noise ratio and clarity of the vessel wall. Images with an image quality ≤2 were excluded from the study. Each imaging location resulted in 4 images with different contrast weightings, which were examined by 1 reader (A.K.) blinded to histology. Areas of hemorrhage were identified using the characteristic combination of signal intensities on the multicontrast MRI (Table 1). The adjacent sternocleidomastoid muscle was used as a reference. These criteria were developed from information on cerebral hemorrhage and our experience in the review of atherosclerotic plaques. Images with multiple contrast weightings could further subdivide hemorrhage by age. These authors highlighted the value of an imaging-based method to study plaque hemorrhage but did not attempt to differentiate between the location of the hemorrhage and the status of the fibrous cap. IH and JLH/T may have different origins and clinical relevance.

<table>
<thead>
<tr>
<th>Hemorrhage Age</th>
<th>Histology: Cell Components</th>
<th>MRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute</td>
<td>Intact red and white blood cells/clotting clusters of macrophages, 1 week</td>
<td>T1: + / T2: ~/ PD: ~ / TOF: +</td>
</tr>
<tr>
<td>Recent</td>
<td>Hemorrhagic debris, intact and degenerating RBCs, fibrin, cholesterol crystals, macrophages, &lt;1 month</td>
<td>T1: + / T2: + / PD: + / TOF: +</td>
</tr>
<tr>
<td>Chronic</td>
<td>Amorphous material, no intact RBCs, necrotic core/matrix, &gt;1 month</td>
<td>T1: ~ / T2: ~ / PD: ~ / TOF: ~</td>
</tr>
</tbody>
</table>

RBC indicates red blood cells; +, hyperintense; o, isointense; and −, hypointense. Interpretation of signal intensity was made with reference to adjacent sternocleidomastoid muscle.

#### Histology Processing and Criteria

After carotid endarterectomy, specimens were fixed in formalin, decalcified, and embedded in paraffin. Samples were sectioned (10 μm thick) every 0.5 to 1.0 mm throughout the length of the specimen and stained (hematoxylin-eosin, Mallory’s trichrome). The slides were independently scored with the quadrant system described above by a reviewer (M.S.F.) who was unaware of the imaging results.

Histology classification of the specimens used criteria established by the AHA’s Committee on Vascular Lesions classification of atherosclerotic lesions. Regions that contained hemorrhage were classified with standard histology criteria (Table 1) used to determine the inflammatory response and healing characteristics of the surrounding tissue. The histological criterion for distinguishing IH from JLH/T is whether the hemorrhage is separated from the lumen by an intact fibrous cap (IH) or is associated with a cap rupture/erosion (JLH/T).

Histology cross sections were also classified according to a modified AHA classification of atherosclerotic lesions (Table 2). By default, sections that contain hemorrhage or thrombus fall into the category of lesion type VI. However, to understand the underlying tissue morphologies of these lesions, we examined lesion type according to the predominant tissues present when hemorrhage and thrombus were excluded. For example, lesions with significant calcification were labeled type VII, and hemorrhages within lipid-rich cores were labeled type IV/V.

#### Matching Between MRI and Histology

Magnetic resonance images and histology sections were independently reviewed and categorized. Landmarks such as the relative distance from the common carotid bifurcation and morphological features such as lumen size and shape, wall size and shape, and prominent calcifications were then used to match images to histology sections. Matched sections were then compared to determine whether MRI correctly identified the presence of IH or JLH/T.
Data Analysis

All calculations were made with SPSS for Windows (version 7.5.1) and R (version 1.8.0). Sensitivity and specificity were calculated on the matched section level by the same approach as in a previously published report. Cohen’s $\kappa$-statistic was computed to quantify the agreement between MRI findings and histology along with an approximate 95% CI based on an assumption of statistical independence between scanned sections. A value of $\kappa \geq 0.7$ was used to indicate a high level of agreement. In addition, the accuracy of MRI for distinguishing IH from JLH/T was computed for correctly identified hemorrhages (true-positives).

The association between type of hemorrhage (JLH/T or IH) and lesion type was analyzed by linear regression in the framework of generalized estimating equations. Generalized estimating equations were used to accommodate the potential statistical dependence among multiple observations per artery. Because the hypotheses of interest concerned type of hemorrhage and lesion types IV and VII, the histological sections were grouped for purposes of analysis. Specifically, sections were grouped into 3 categories: (1) type IV or V, (2) type VII, and (3) all other lesion types or combinations of types (including, for example, 15 sections with mixed type IV and VII). For each patient and within each category, the percent of sections with IH was calculated after sections with no hemorrhage were dropped. The percentage can be treated as a continuous variable. A linear regression (using generalized estimating equations) was performed with this percentage as the dependent variable and the lesion category as the independent variable (eg, types IV/V versus VII, represented by dummy variables).

Results

Of the 26 subjects enrolled in the study, 2 were excluded from analysis because of poor image quality in all cross-sectional images of the examination. For the remaining 24 subjects, 8 cross sections were eliminated because of insufficient image quality, which left 190 matched image sets (760 total images). Within these 190 sections, histological analysis found 140 that contained hemorrhage, of which 100 contained IH only and 40 contained JLH/T. Table 3 shows the corresponding readings by MRI for these 190 sections. Excellent agreement is observed with $\kappa = 0.82$ (95% CI 0.74 to 0.89).

From Table 3, one can compute sensitivity and specificity for detecting sections that contained any plaque hemorrhage.
by combining the 2 hemorrhage categories to produce the standard 2-by-2 table. This shows that any hemorrhage is detected with a sensitivity of 96% and a specificity of 82%. A similar analysis showed that MRI is able to detect JLH/T with a sensitivity of 88% and a specificity of 98%. These data indicate an accuracy of 96% for distinguishing JLH/T from IH in correctly identified hemorrhages, with $\kappa = 0.91$.

Finally, to investigate the association between underlying lesion type, as defined by AHA criteria, and the occurrence of hemorrhage, an analysis (generalized estimating equation) that used only the histological data was performed. IH and JLH/T differed significantly in the distribution of types IV/V, VII, and “other” lesions in the same sections ($P = 0.004$; Figure 4). JLH/T was associated in 70% of all sections with underlying type VII (calcified lesion), in 21% with underlying lesion type IV/V lesion (uncomplicated atheromas, fibroatheroma), and in 9% with other lesions. Fifty-five percent of sections with IH were associated with type IV/V lesion, 34% with type VII lesion, and 11% with other lesion. A post hoc analysis showed that the different proportions of type IV/V (combined) versus type VII lesions was responsible for the highly significant difference in distributions of IH versus JLH/T. With a Bonferroni correction for 3 post hoc comparisons, significant differences in the proportion of type IV/V versus type VII lesions ($P < 0.001$) were found. No significant differences were found between type IV/V versus other lesions ($P = 1.0$) and proportions of type VII versus other lesions ($P = 0.4$).

Discussion

Previous studies have shown that MRI is able to identify recent IH with high sensitivity and specificity$^{16}$ and to accurately determine hemorrhage age.$^{24}$ The present study demonstrates the ability of in vivo multicontrast-weighted MRI to further characterize the location of hemorrhage (intraplaque versus juxtaluminal) of advanced atherosclerotic lesions in the human carotid artery. The clinical value is apparent in that previously published studies suggest that IH without fibrous cap rupture is not associated with patient symptoms,$^{10,30}$ whereas JLH/T indicates an erosion, ulceration, or rupture, each of which is recognized as a marker of unstable plaque.$^{20,27,30}$

MRI can identify and accurately differentiate between IH and JLH/T as verified by the high $\kappa$-value (0.82). CIs for $\kappa$-values (0.74 to 0.89) were calculated on the basis of the assumption of independence between cross sections. Given that more than 1 cross section was utilized for analysis from each plaque, a certain interdependence between cross sections may exist. We have previously shown that interdependence between sections is relatively low ($\kappa = 0.48^{16}$) and can therefore conclude that the true CI is only slightly wider than that quoted.

In the present study, multiple sets of contrast criteria were used to detect plaque hemorrhage, with subcategories of acute, recent, or chronic.$^{24}$ The need for different criteria for different ages of hemorrhage arises because the signal intensity of hemorrhage is dependent on the structure of hemoglobin and its oxidation state.$^{26}$ We attribute the high sensitivity (96%) and specificity (82%) for overall hemorrhage detection to the recognition of changing signal features with age and the use of multiple contrast weightings. The use of criteria for different ages further suggests that MRI can be used to study the combined implications of both hemorrhage location (IH versus JLH/T) and hemorrhage age in atherosclerotic plaque.

Misclassification of IH and JLH/T is attributable to 2 factors: (1) poor image quality and (2) spatial resolution. An image quality of 3.0 indicates a marginal signal-to-noise ratio where wall structures are identifiable but lumen and outer wall boundaries are partially obscured, which complicates the proper assessment of the luminal surface and therefore the classification of IH versus JLH/T. Other potential sources for misclassification are insufficient spatial resolution, such as a very thin layer of fibrous tissue covering an IH that might be misclassified as JLH/T or a very small lumen surface that might complicate the proper assessment of the lumen surface status.

The potential for different intraplaque tissues to have similar contrast characteristics can affect the accuracy of magnetic resonance detection. For example, both calcification and chronic hemorrhage produce hypointense signals in all 4 contrast weightings. Differentiation between the 2 requires examination of the borders of the hypointense signal. A sharp and well-defined edge on PD/T2-weighted image confirmed by a matching hypointense signal on TOF and

### TABLE 2. Modified AHA Classification of Atherosclerotic Plaque

<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
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<tbody>
<tr>
<td>I–II</td>
<td>Near-normal vessel-wall thickness, no calcification</td>
</tr>
<tr>
<td>III</td>
<td>Diffuse intimal thickening or small eccentric plaque with no calcification</td>
</tr>
<tr>
<td>IV/V</td>
<td>Plaque with lipid or necrotic core surrounded by fibrous tissue with possible calcification</td>
</tr>
<tr>
<td>VI</td>
<td>Complex plaque with possible surface defect, hemorrhage, or thrombus</td>
</tr>
<tr>
<td>VII</td>
<td>Calcified plaque</td>
</tr>
<tr>
<td>VIII</td>
<td>Fibrotic plaque with possible small calcifications</td>
</tr>
</tbody>
</table>

### TABLE 3. Content by Section of IH and JLH/T: MRI vs Histology

<table>
<thead>
<tr>
<th>MRI</th>
<th>IH</th>
<th>JLH/T</th>
<th>None</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>IH</td>
<td>93</td>
<td>3</td>
<td>9</td>
<td>105</td>
</tr>
<tr>
<td>JLH/T</td>
<td>3</td>
<td>35</td>
<td>0</td>
<td>38</td>
</tr>
<tr>
<td>None</td>
<td>4</td>
<td>2</td>
<td>41</td>
<td>47</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>40</td>
<td>50</td>
<td>190</td>
</tr>
</tbody>
</table>

$\kappa$-Value (95% CI) $= 0.82$ (0.74 – 0.89).
T1-weighted image is consistent with calcification, whereas an ill-defined edge on a PD/T2-weighted image confirmed by a TOF/T1-weighted image is consistent with chronic hemorrhage. Thick fibrous caps may also present a juxtaluminal band of low signal on TOF and may be misinterpreted as calcification. However, examination of the T1-, PD-, and T2-weighted images permits distinction between calcification (hypoointense) and thick fibrous cap (isointense). Conversely, calcific nodules that extend into the lumen are often masked on black-blood sequences because of low signal intensity, which makes a bright-blood sequence (TOF) mandatory. Therefore, lesions that appear to be calcified (type VII) should be meticulously examined for the presence of surface irregularities and defects to identify the presence of juxtaluminal calcification.

The second question addressed by the present study was whether a particular set of tissues is found near either JLH/T or IH. Our histology examination showed that tissues underlying hemorrhages could primarily be classified as either lipid-rich with large necrotic cores (type IV/V) or heavily calcified fibrous lesion (type VII). Both lesion types can produce hemorrhagic incidents. Milei et al reported that tissues adjacent to large lipid cores are often vascularized with heterogeneous neovessels. An increase in the amount of lipid in the core, mechanical stress, and an overproduction of oxygen free radicals by macrophages can lead to the breakdown of new vessels, creating IH. The results of the present study demonstrate that calcifications, historically considered benign, might in fact be a causative factor of erosion and ulceration.

In summary, the ability to detect and localize hemorrhage suggests that MRI can be used to examine the role of hemorrhage in the progression of lesions over time. Furthermore, in the present investigation, a potential link between hemorrhage and underlying lesion type is suggested by analysis of the histology data. Previous work by Cai et al showed that MRI can also noninvasively document carotid lesions according to a modified AHA classification. In principle, MRI can now be used to prospectively examine the interrelationships of hemorrhage and lesion-type progression.

Conclusions

Analysis of the underlying AHA lesion types suggests potential differences in the origin of intraplaque and juxtaluminal hemorrhage. Further study with in vivo serial lesion assessment is needed to better understand the mechanisms involved in the development of different types of plaque hemorrhage. The results of the present study demonstrate that multicontrast-weighted MRI can distinguish IH and JLH/T with a high degree of accuracy in vivo. Therefore, MRI provides a valuable tool for prospectively examining the pathophysiology of plaque hemorrhage types. Furthermore, serial studies with MRI will permit assessment of the relationship between hemorrhage type, plaque progression, and the development of ischemic complications.

Acknowledgment

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References


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