Presence of Intraplaque Hemorrhage Stimulates Progression of Carotid Atherosclerotic Plaques
A High-Resolution Magnetic Resonance Imaging Study

Norihide Takaya, MD, PhD; Chun Yuan, PhD; Baocheng Chu, MD, PhD; Tobias Saam, MD; Nayak L. Polissar, PhD; Gail P. Jarvik, MD, PhD; Carol Isaac, RVT; Judith McDonough, BS; Cynthia Natiello, RN; Randy Small, HT; Marina S. Ferguson, MT; Thomas S. Hatsukami, MD

Background—Previous studies suggest that erythrocyte membranes from intraplaque hemorrhage into the necrotic core are a source of free cholesterol and may become a driving force in the progression of atherosclerosis. We have shown that MRI can accurately identify carotid intraplaque hemorrhage and precisely measure plaque volume. We tested the hypothesis that hemorrhage into carotid atheroma stimulates plaque progression.

Methods and Results—Twenty-nine subjects (14 cases with intraplaque hemorrhage and 15 controls with comparably sized plaques without intraplaque hemorrhage at baseline) underwent serial carotid MRI examination with a multicontrast weighted protocol (T1, T2, proton density, and 3D time of flight) over a period of 18 months. The volumes of wall, lumen, lipid-rich necrotic core, calcification, and intraplaque hemorrhage were measured with a custom-designed image analysis tool. The percent change in wall volume (6.8% versus 0.15%; \( P < 0.009 \)) and lipid-rich necrotic core volume (28.4% versus 5.2%; \( P = 0.001 \)) was significantly higher in the hemorrhage group than in controls over the course of the study. Furthermore, those with intraplaque hemorrhage at baseline were much more likely to have new plaque hemorrhages at 18 months compared with controls (43% versus 0%; \( P = 0.006 \)).

Conclusions—Hemorrhage into the carotid atherosclerotic plaque accelerated plaque progression in an 18-month period. Repeated bleeding into the plaque may produce a stimulus for the progression of atherosclerosis by increasing lipid core and plaque volume and creating new destabilizing factors. (Circulation. 2005;111:2768-2775.)

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

The progression of atherosclerosis is a complex and often longstanding phenomenon. Many studies have shown that inflammation and the subsequent immune response contribute to atherogenesis. The recruitment of inflammatory cells in atherosclerotic lesions is a constitutive phenomenon throughout the process of plaque growth.1,2 Intraplaque hemorrhage is commonly observed in atherosclerotic plaques and is considered by some to be caused by rupture of plaque neovasculature. These microvessels are fragile because of lack of support by smooth muscle cells and focal discontinuity of the endothelial lining.3

In a recent histopathological study of coronary artery specimens, Kolodgie et al4 suggested that intraplaque hemorrhage may represent a potent atherogenic stimulus by contributing to the deposition of free cholesterol, macrophage infiltration, and enlargement of the necrotic core. Immunohistochemical staining with the antibody to glycoporphin A, a protein specific to erythrocyte membranes, was strongly associated with the size of the necrotic core and degree of macrophage infiltration. They also noted that rabbit lesions with induced intramural hemorrhage had significantly greater lipid content than control lesions without hemorrhage. Other investigators have noted that erythrocyte membranes contain more free cholesterol than any other cell in the body.5

To test the hypothesis that intraplaque hemorrhage is a predisposing factor in progression of human atherosclerosis, an imaging tool is needed that can serially examine the morphological and compositional characteristics of the lesion over time. High-resolution MRI is ideally suited for this purpose because it is noninvasive and has proven capability for distinguishing tissue characteristics.6–9 Our laboratory recently demonstrated that multicontrast MRI can accurately...
detect the presence and age of carotid intraplaque hemorrhage. Furthermore, MRI is capable of precisely quantifying overall lesion size and plaque compositional features such as the lipid-rich necrotic core. Thus, this imaging tool provides a unique opportunity to examine the temporal relationship between baseline plaque characteristics and subsequent progression in lesion size and composition.

The aim of this study was to test the hypothesis that intraplaque hemorrhage, as detected by high-resolution MRI, is associated with greater progression in both lipid-rich necrotic core and plaque volume over a period of 18 months.

**Methods**

**Study Population**

Cases and controls were selected from an ongoing prospective serial carotid MRI study referred to as PRIMARI. Subjects for PRIMARI were recruited from the diagnostic vascular ultrasound laboratories at the University of Washington Medical Center and Veterans Affairs Puget Sound Health Care System after informed consent was obtained. The study procedures and consent forms were reviewed and approved by each site’s institutional review board. Inclusion criteria for the PRIMARI study included (1) 50% to 79% carotid stenosis by duplex ultrasound examination and (2) asymptomatic status with regard to carotid artery disease within the 6 months before enrollment in PRIMARI. Exclusion criteria included (1) prior carotid endarterectomy (CEA) on the side of the index carotid artery, (2) prior neck irradiation, and (3) contraindication for MRI. Subjects undergo bilateral carotid artery MRI every 18 months. At the time of analysis, 142 subjects had at least 2 MRI scans (baseline and 18 months). Among the 142 patients (284 arteries) available, 66 arteries were excluded because of marginal image quality, insufficient plaque size, or absence of the common carotid bifurcation in the field of view. In this serial MRI study, the common carotid artery on the index side was used as an internal fiducial marker to match cross-sectional locations between MRI examinations. In some cases, the bifurcation on the contralateral, nonindex side occurred at a different axial level, outside the field of view. Four arteries with total occlusion were excluded, as were 11 arteries with MRI evidence of fibrous cap rupture or ulceration with mural thrombus formation. The latter were excluded to focus the analysis of this study on the association between intraplaque hemorrhage (presumably from rupture of plaque neovascularure) and plaque progression.

Images from the baseline MRI examination were first reviewed to identify cases and controls blinded to the follow-up MR studies. Among the 203 arteries remaining, 14 arteries (99 cross-sectional locations) demonstrated clear MRI evidence of early or recent intraplaque hemorrhage. Fifteen arteries (103 cross-sectional locations) with comparable baseline plaque volume that clearly had no evidence of early, recent, or chronic intraplaque hemorrhage were selected as controls. The baseline and the 18-month follow-up MRI examinations were used to compare cases and controls for progression in overall plaque volume and plaque constituents. Two patients underwent CEA at 2 months and 1 year after the second MRI examination, and detailed histological analysis was performed for comparison with the MRI findings with the use of a previously published protocol.

**Baseline Clinical and Laboratory Data**

Before the baseline MRI examination, study subjects were asked to complete a detailed health questionnaire and physical examination to assess risk factors for atherosclerosis (hypertension, diabetes, smok-
ing, family history), history of coronary artery and peripheral vascular disease, current medications, and body mass index. Lipid measurements were performed on fasting whole plasma. Standard enzymatic methods were used to determine levels of total cholesterol, triglycerides, and HDL cholesterol on an Abbott Spectrum analyzer. \textsuperscript{13,16} LDL cholesterol was calculated by the Friedewald equation.

**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 (time of flight [TOF], T1, proton density [PD], and T2) of the carotid arteries centered at the common carotid bifurcation on the index side. \textsuperscript{8} MRI parameters were as follows: T1-weighted (T1W): double inversion recovery, black blood, 2D fast spin-echo, repetition time (TR)/effective echo time (TE) = 800/10 ms, echo train length = 8; PD-weighted (PDW) and T2-weighted (T2W): fast spin echo, cardiac gated, TR = 3 or 4 cardiac R-R intervals, effective TE = 20 ms for PDW and 40 ms for T2W, echo train length = 6; and 3D TOF: TR/TE/flip angle = 23.8 ms, 800/10 ms, flip angle 25°. Fat suppression was used for T1W, PDW, and T2W images to reduce signals from subcutaneous fat. Images were obtained with a field of view of 13 to 16 cm, matrix size of 256, slice thickness of 2 mm, and 2 signal averages. Inter-slice spacing was 0 mm for T1W, PDW, and T2W and -1 mm for 3D TOF (1 mm overlapping between adjacent slices). The longitudinal coverage of each carotid artery was 24 mm (12 slices) for T1W, 30 mm (15 slices) for PDW and T2W, and 40 mm (40 slices) for 3D TOF. A zero-filled Fourier transform was used to reduce pixel size (0.25×0.25 to 0.31×0.31 mm\(^2\)) and minimize partial-volume artifacts.

**MRI 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 boundaries. \textsuperscript{17} Images with an image quality ≤ 2 were excluded from the study. After identifying cases and controls, 1 reviewer (N.T.), who was blinded to time points, examined all of the MR images.

Area measurements of the lumen, outer wall, and tissue components (lipid-rich necrotic core, calcium, and hemorrhage) were obtained with a custom-designed imaging analysis tool (QVAS). \textsuperscript{13} Wall area was calculated as the difference between outer wall and lumen area. The carotid bifurcation was used as the landmark for matching the 4 different contrast weightings of the 2 image sets at 2 time points. Only matched locations covered by both time points were used for analysis.

Areas of hemorrhage, lipid-rich necrotic core, and calcification were identified as described previously, \textsuperscript{10} with modification in terminology, in which the terms type I and type II rather than fresh and recent were used to describe the age of intraplaque hemorrhage. Briefly, fresh intraplaque hemorrhage (type I) appears as a hyperintense signal on T1W and TOF images and as an isointense signal on PD/T2W images compared with the signal of the adjacent stenomecoidomastoid muscle. Recent hemorrhage (type II) is identified by a hyperintense signal on all 4 contrast weightings. The lipid-rich necrotic core without hemorrhage is identified by a hypointense to isointense signal on T1W and PDW images, a varied signal on T2W images, and an isointense signal on TOF. Calcium is characterized by well-defined hypointense signal areas in all 4 contrast weightings. Figure 1 shows representative images of a lipid-rich necrotic core without hemorrhage, a necrotic core containing type I hemorrhage, and a necrotic core containing type II hemorrhage.

**Histology Processing**

After the second MRI scan, 2 patients underwent CEA. The 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 evaluated by a reviewer (M.S.F) who was unaware of the imaging results.

**Immunohistochemical staining of glycophorin A (1:500 dilution; Daco) and Mallory’s iron stain were performed.** \textsuperscript{4,18} The age of hemorrhage was classified as previously described. \textsuperscript{10}

**Statistical Analysis**

Data were expressed as mean±SD. Percent changes in measurements over time were calculated for individual subjects. The Student paired \(t\) test was used for comparison of means within the same group, and the Student unpaired \(t\) test was used for comparison between 2 groups. Linear regression was used to compare percent change between groups while controlling for potentially confounding variables. Categorical variables were presented as counts and percentages per category and compared by means of Fisher exact test. All calculations were made with SPSS 12.0 for Windows. Statistical significance was defined as a value of \(P<0.05\). Volumes were measured including all slices available with American Heart Association grade ≥3. Volumes were then normalized for varying numbers of slices by adjustment to yield a volume for 10 slices: adjusted volume=observed volume×(10 slices/observed number of slices).

**Patient Characteristics**

Baseline clinical characteristics of cases and controls are presented in Table 1. Baseline characteristics of the 2 groups

**TABLE 1. Baseline Characteristics**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hemorrhage Group ((n=14))</th>
<th>Control Group ((n=15))</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>71.1±11.1</td>
<td>70.5±8.5</td>
<td>0.9</td>
</tr>
<tr>
<td>Interval between scans(* )</td>
<td>17.8 (16.6–19.6)</td>
<td>18.0 (16.4–19.5)</td>
<td>0.5</td>
</tr>
<tr>
<td>Male sex, %</td>
<td>92.9</td>
<td>86.7</td>
<td>0.5</td>
</tr>
<tr>
<td>Body mass index, kg/m(^2)</td>
<td>26.9±4.5</td>
<td>26.9±4.9</td>
<td>1</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>92.9</td>
<td>80</td>
<td>0.6</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>14.3</td>
<td>33.3</td>
<td>0.4</td>
</tr>
<tr>
<td>Smoking, %</td>
<td>50</td>
<td>53.3</td>
<td>1</td>
</tr>
<tr>
<td>History of CAD, %</td>
<td>64.3</td>
<td>53.3</td>
<td>0.7</td>
</tr>
<tr>
<td>Medication, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Statin</td>
<td>64.3</td>
<td>80</td>
<td>0.4</td>
</tr>
<tr>
<td>Aspirin</td>
<td>57.1</td>
<td>73.3</td>
<td>0.5</td>
</tr>
<tr>
<td>ACE inhibitor</td>
<td>42.9</td>
<td>46.7</td>
<td>1</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>177.1±38.8</td>
<td>179.7±37.7</td>
<td>0.9</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>78.4±31.5</td>
<td>73.9±26.5</td>
<td>0.7</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>45.3±9.6</td>
<td>40.4±12.44</td>
<td>0.3</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>119.1±57.6</td>
<td>181.1±110.2</td>
<td>0.07</td>
</tr>
</tbody>
</table>

\(\text{*Mean number of months (range).}\)

**TABLE 2. Normalized Volume Measurements at Baseline**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hemorrhage Group ((n=14))</th>
<th>Control Group ((n=15))</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumen, mm(^3)</td>
<td>559.4±231.5</td>
<td>475.8±164.5</td>
<td>0.1</td>
</tr>
<tr>
<td>Wall, mm(^3)</td>
<td>1197.6±264.9</td>
<td>996.1±302.1</td>
<td>0.07</td>
</tr>
<tr>
<td>Lipid-rich necrotic core, mm(^3)</td>
<td>296.9±118.3</td>
<td>199.5±88.6</td>
<td>0.02</td>
</tr>
<tr>
<td>Intraplaque hemorrhage, mm(^3)</td>
<td>196.3±112.5</td>
<td>0±0*</td>
<td></td>
</tr>
<tr>
<td>Calcium, mm(^3)</td>
<td>24.4±28.2</td>
<td>27.7±25.4</td>
<td>0.7</td>
</tr>
</tbody>
</table>

\(\text{Values are mean±SD unless indicated otherwise. CAD indicates coronary artery disease.}\)

\(\text{\textsuperscript{*By design and selection of subjects.}}\)
TABLE 3. Percent Change of Volume and Wall Thickness

<table>
<thead>
<tr>
<th></th>
<th>Hemorrhage Group (n=14)</th>
<th>Control Group (n=15)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumen volume</td>
<td>−8.5±12.2</td>
<td>1.5±7.9</td>
<td>0.014</td>
</tr>
<tr>
<td>Wall volume</td>
<td>6.8±7.9</td>
<td>−0.15±5.1</td>
<td>0.009</td>
</tr>
<tr>
<td>Outer wall volume</td>
<td>1.5±6.2</td>
<td>0.2±4.6</td>
<td>0.5</td>
</tr>
<tr>
<td>Lipid-rich necrotic core volume</td>
<td>28.4±29.7</td>
<td>−5.2±17.3</td>
<td>0.001</td>
</tr>
<tr>
<td>Calcium volume</td>
<td>0.3±38.4</td>
<td>3.4±26.1</td>
<td>0.8</td>
</tr>
<tr>
<td>Maximum wall thickness</td>
<td>8.3±11.3</td>
<td>−3.2±10.7</td>
<td>0.009</td>
</tr>
<tr>
<td>Minimum wall thickness</td>
<td>3.9±21.3</td>
<td>−5.6±23.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Mean wall thickness</td>
<td>8.5±8.8</td>
<td>−1.6±4.74</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Values are mean±SD.

were not significantly different at the 0.05 level.

Baseline measurements of wall, lumen, and plaque components are shown in Table 2. The baseline volume for lumen, wall, and calcification was similar in both groups. Although the volume of the lipid-rich necrotic core at baseline was significantly larger in the hemorrhage group than in the control group, this could be attributed to the inclusion of hemorrhage volume in the lipid-rich necrotic core volume.

The mean observational periods of both groups were 17.8 months (range, 16.6 to 19.6 months) and 18.0 months (range, 16.4 to 19.5 months) for the hemorrhage and control groups, respectively. There was no significant difference in the observation period between the 2 groups (P=0.5).

Two patients in the hemorrhage group underwent CEA during the course of the study. One of the CEA patients developed transient hemispheric ischemia in the distribution of the index carotid artery 3 weeks before surgery, and the other patient had asymptomatic progression of carotid stenosis to >80%. In the control group, 1 patient described an episode of amaurosis fugax on the side of the index carotid artery during the observational period. However, CEA was not performed because of prohibitive cardiac risk to surgery.

Percent Volume Change

The percent change in wall volume (6.8±7.9% versus −0.15±5.1%; P=0.009) and lipid-rich necrotic core volume (28.4±29.7% versus −5.2±17.3%; P=0.001) was significantly higher in the hemorrhage group than in the control group (Table 3). Lumen volume was reduced in the hemorrhage group compared with the control group (−8.5±12.2% versus 1.5±7.9%; P=0.001). Maximum and mean wall thickness increased significantly in the hemorrhage group versus the control group (8.3±11.3% versus 3.2±10.7%; P=0.009; 8.5±8.8% versus −1.6±4.7%; P=0.001). Figure 2 is an example of intraplaque hemorrhage, lumen narrowing, and wall increase over the 18-month follow-up period.

In the hemorrhage group, wall volume and lipid-rich necrotic core volume increased despite statin therapy. However, the percent change in volume was smaller in the subgroup treated with statins compared with those not receiving statin therapy (5.9±6.3% versus 8.4±10.9% for wall volume and 25.4±14.7% versus 33.9±48.7% for lipid-rich necrotic core volume, respectively), but these differences were not significant (P=0.6).

Hemorrhage Signal Intensity Change

Baseline examination of the 29 patients produced 80 MR slices (83 locations) of hemorrhage. Type I hemorrhage was present in 6 slices (7.5%), and type II hemorrhage was observed in 71 slices (88.8%). Three slices (3.8%) contained both type I and type II hemorrhages in the same slice level. Interestingly, at the 18-month examination, 78 locations (94.0%) demonstrated signal intensity, indicating that the hemorrhage stage remained unchanged. Eight locations (88.9%) of type I hemorrhage and 70 locations (94.5%) of type II hemorrhage remained constant. Figure 3 shows a representative case of type II hemorrhage that retained the same hyperintense signal in all 4 weightings over a period of 18 months. CEA was performed in this case 1 year after the second MRI examination, and the specimen was processed and serially sectioned. Histology examination demonstrated recent hemorrhage (type II) in the same regions as in the matched MR images (Figure 3C). Those areas also had abundant cholesterol crystals and showed extensive staining for glycoprophin A within the necrotic core (Figure 3D, 3E).

New Hemorrhage

Six of the 14 patients (43%) with intraplaque hemorrhage at baseline developed intraplaque hemorrhage in new locations within the plaque during the 18-month study period, whereas no new incidents of hemorrhage were detected in the control group (P=0.006, Fisher exact test) (Table 4). In location-based analysis, new hemorrhage was observed in 12 sites (12.6%). Further evidence of repeated intraplaque hemor-

Figure 2. Representative T1-weighted images of progression of atherosclerosis with intraplaque hemorrhage in right carotid artery. Each column presents matched cross-sectional locations in carotid artery from baseline MRI (A) and MRI obtained 18 months later (B). Lumen area was decreased, and wall area was increased in each section at second examination. CCA indicates common carotid artery; Bif, bifurcation; ICA, internal carotid artery; and ECA, external carotid artery.
rhage is provided by the increase in volume of intraplaque hemorrhage over the 18-month period, from 146.6±118.9 to 181.5±161.8 mm³, but the increase was not statistically significant (P=0.06).

Arteries that exhibited evidence of repeated intraplaque hemorrhage were also associated with significant increase in percent change of mean wall thickness (14.8% versus 3.7%; P=0.013) as well as a significant decrease in percent change of lumen volume (−16.4% versus −2.5%; P=0.013) compared with plaques without evidence of repeated intraplaque hemorrhage. The change in wall and lipid-rich necrotic core volume absent the volume of new hemorrhage was still significantly greater than change in the control group (5.7% versus −0.2%, P=0.021; 24.0% versus −5.2%, P=0.002).

In addition, a comparison of intraplaque hemorrhage patients without new hemorrhage during follow-up (n=8) with controls (n=15) also showed that the percent changes of wall and lipid-rich necrotic core volumes were greater than the changes of the control group (5.3% versus −0.2%, P=0.049; 19.0% versus −5.2%, P=0.03).

Histological findings from the excised carotid specimens provided further evidence of repeated intraplaque hemorrhage. Figure 4 shows extensive microvasculature with surrounding intraplaque hemorrhage of an earlier phase than that deep within the core. Mallory’s stain for iron was used on sections from each level of the 2 excised specimens to provide confirmation of red cell breakdown and erythropagocytosis. Iron, in the form of inclusions, was predominantly found in macrophages in tissues peripheral to the necrotic core.

**Discussion**

To our knowledge, this study is one of the first to demonstrate an association between baseline plaque compositional characteristics, specifically intraplaque hemorrhage, and progression of human atherosclerosis in a prospective, serial fashion with the use of high-resolution MRI. In this study carotid plaques with evidence of early or recent intraplaque hemorrhage at baseline were significantly more likely to increase in overall wall volume and lipid-rich necrotic core volume and more likely to decrease in lumen size over an 18-month period. Furthermore, patients with intraplaque hemorrhage at baseline had a greater probability of repeated carotid intraplaque hemorrhage.

Findings from this study are consistent with the suggestion by Kolodgie et al4 that intraplaque hemorrhage may stimulate atherogenic activity by being a source of free cholesterol and macrophage activation. Other studies based on histopathological examination of excised plaques also suggest that intraplaque hemorrhage may be a powerful instigator of progression.19–21 Kockx et al20 reported that intraplaque microhemorrhages initiated phagocytosis of platelets and erythrocytes, leading to activation of inducible NO synthase expressing foam cells. They also found that clusters of macrophages located near microvessels appeared to have ingested red cell fragments. Yuan et al21 noted that erythropagocytosis enhances the capacity of macrophages to oxidize LDL, leading to conversion of LDL to a form that promotes lipid accumulation in macrophages. This longitudinal, in vivo study corroborates these in vitro findings by showing not only an increase in the total volume of the atherosclerotic vessel

---

**TABLE 4. Proportion of Patients With New Incidents of Intraplaque Hemorrhage During 18-Month Follow-up**

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>New Hemorrhage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Hemorrhage group</td>
<td>14</td>
<td>100</td>
</tr>
<tr>
<td>Control group</td>
<td>15</td>
<td>100</td>
</tr>
</tbody>
</table>

P=0.006 for comparison of new hemorrhage percentage between control group and intraplaque hemorrhage group, Fisher exact test.
In contrast, the control group did not exhibit an increase in plaque volume (−0.15±5.1%) and showed a decrease in the lipid-rich necrotic core volume (−5.2±17.3%). These results suggest that plaques without hemorrhage are less active or perhaps that statin treatment (73.3% of control subjects) truly did abolish progression of atherosclerosis, as previously described in studies by Corti et al.22 and Nissen et al.23 However, because plaques in the hemorrhage group progressed rapidly despite treatment with statins (64.3% of subjects), this may indicate that the physical increase in plaque volume and cholesterol supply by repeated intraplaque hemorrhage may exceed the effect of statin therapy. On the other hand, statin-treated plaques in the hemorrhage group had a smaller percent change of wall (5.9±0.15%) and showed a decrease in the lipid-rich necrotic core (5.2±14.7%) volume, which suggests that statin therapy may suppress the progression of plaques even in the presence of hemorrhage. However, neither change in these volumes was statistically significant. Further study with a larger group of patients with intraplaque hemorrhage is needed for this aspect of the study.

Findings from this study also suggest the importance of repeated hemorrhage into the plaque as an additional mechanism of progression. New hemorrhages occurred in at least 43% of patients with previous intraplaque hemorrhage, whereas no new hemorrhages appeared in the control group. Plaques with MRI evidence of repeated intraplaque hemorrhage were associated with a significant increase in mean wall thickness (14.8% versus 3.7%; P=0.013) and decrease in lumen volume (−16.4% versus −2.5%; P=0.013) compared with plaques without evidence of repeated intraplaque hemorrhage. This difference suggests that repeated intraplaque hemorrhage may be a marker for plaque instability and may be a contributing factor toward a more rapid progression of atherosclerosis. The disruption of thin-walled microvessels with discontinuous endothelium is believed to be a cause of intraplaque hemorrhage. These fragile microvessels are implicated in repeated intraplaque capillary rupture.3,20 Indeed, close histological examination of the necrotic core of the excised CEA specimens showed extensive microvasculature with surrounding intraplaque hemorrhage of an earlier phase than that deep within the core (Figure 4). On the other hand, the change in wall and lipid-rich necrotic core volume absent the volume of new hemorrhage was still significantly greater than the change of the control group. Furthermore, intraplaque hemorrhage patients without new hemorrhage during follow-up also had greater change in wall and lipid-rich necrotic core volumes than the controls. These data suggest that increase in wall and lipid-rich necrotic core volume may result not only from the influx of new hemorrhage but also from other mechanisms, possibly from increase in plaque cholesterol and activation of macrophages, as suggested by Kolodgie et al.4

Our previously published work established the criteria for the identification of the stages of intraplaque hemorrhage with the use of multicontrast MRI.10,12 The concept of the change in signal intensity of intraplaque hemorrhage was based on the detection and staging of cerebral hemorrhage with the use of multicontrast MR images. The stages of hemorrhage are based on red blood cell content and the oxygenation of hemoglobin. The criteria used to classify early subacute cerebral hemorrhage were applied to the carotid intraplaque hemorrhage and given the term fresh. Morphologically, this includes the presence of intact red blood cells, macrophage and inflammatory cell infiltrate, and evidence of early thrombus formation, which by MRI appears hyperintense on TOF and T1W images. The second category, termed recent, used the late subacute criteria from brain hemorrhage, which includes hemorrhagic debris, intact and degenerating red blood cells, and the increased interstitial fluid associated with inflammation and early organization. These latter components combine to create hyperintense signal patterns in all contrast weightings. Our use of the terms type I and type II rather than fresh and recent, respectively, is based on an
evolving understanding of the distinct time course of intraplaque hemorrhage in the carotid artery. This study demonstrates that in the majority (94%) of locations with intraplaque hemorrhage at baseline, the signal intensity associated with hemorrhage remained unchanged at the 18-month time point. MRI studies of intracranial hemorrhage demonstrate a rapid evolution of signal intensity changes within days after the hemorrhagic event that are related to the oxidative state of hemoglobin and the eventual lysis of red blood cells. Erythrocyte lysis and hemoglobin oxygenation are heavily dependent on local factors such as oxygen tension and pH. Our results suggest that atherosclerotic intraplaque hemorrhage may have a longer course for change due to inherent differences in the tissue environment within the necrotic core. Intraplaque hemorrhage may attract macrophages that eventually become trapped within the core and are unable to survive. Our histological assessment showed that macrophages with iron inclusions were found in fibrous areas around the core. The infrequency of macrophage iron-laden cells in the core indicates that the degradation of hemorrhage may be slow because of reduced activation of macrophages. Alternatively, repeated hemorrhage into the same plaque region may account for the absence of expected signal intensity changes over the 18-month interval between scans. Because our study included a relatively small number of type I hemorrhage patients at baseline, further analysis using more patients will be needed to solidify our understanding of the signal intensity change of type I hemorrhage. Additionally, to establish the time course of the signal intensity changes of intraplaque hemorrhage, a more frequent schedule of MRI examinations after the intraplaque hemorrhage event is needed.

Finally, findings from this study also provide insight into the remodeling of the carotid atherosclerosis. Lumen stenosis progressed more rapidly in the hemorrhage group than in the control group. Percent change of lumen volume was −8.5 ± 12.2% for the hemorrhage group and 1.5 ± 7.9% for control subjects (P = 0.014). However, in comparison, the outer wall volume increased by only a small amount among cases compared with controls (1.5 ± 6.2% versus 0.2 ± 46%, respectively; P = NS), suggesting that positive remodeling played little role in the increase in wall volume associated with intraplaque hemorrhage. Both of these trends are consistent with previous histopathological studies indicating that intraplaque hemorrhage has a significant correlation primarily with increased luminal narrowing.

As demonstrated in this study, the clinical implications of serial MR examinations are significant. MRI can give information not only about the change in lumen size and plaque volume but also about plaque components, inflammation, and fibrous cap status noninvasively. Findings from these studies may provide new insight into the pathophysiology of atherosclerosis and permit direct assessment of the effect of pharmacological therapy.

Limitations of Study
At baseline, the lipid-rich necrotic core volume was larger in the hemorrhage group than in the controls, which was anticipated, given that intraplaque hemorrhage is typically admixed within the necrotic core of the plaque. Of note, however, when we controlled for baseline volume of lipid-rich necrotic core and baseline wall volume, the association of intraplaque hemorrhage with increased plaque volume was still statistically significant (P = 0.009). It is important to remember that however intriguing these results may be, this study focused only on the carotid artery, and therefore extrapolation to other vascular beds is not possible at this time.

Also of note, 56 (19.7%) of the 284 arteries available for analysis were excluded because of marginal image quality. New technological developments in hardware and image acquisition techniques should improve overall image quality and reduce the number of excluded cases.

Conclusions
This in vivo MRI progression study demonstrates that hemorrhage into the carotid atherosclerotic plaque is significantly associated with progression in wall and lipid-rich necrotic core size and a decrease in lumen volume. Furthermore, lesions that had intraplaque hemorrhage at baseline had a greater probability of repeated intraplaque hemorrhage at 18 months. We propose that repeated hemorrhage and the resulting consequences of cholesterol released from trapped erythrocytes and activated macrophages may be important factors in the rapid progression of carotid atherosclerosis.

Acknowledgments
This study was supported by National Institutes of Health grants R01 HL61851, R01 HL073401, and HL67406.

References


Presence of Intraplaque Hemorrhage Stimulates Progression of Carotid Atherosclerotic Plaques: A High-Resolution Magnetic Resonance Imaging Study
Norihide Takaya, Chun Yuan, Baocheng Chu, Tobias Saam, Nayak L. Polissar, Gail P. Jarvik, Carol Isaac, Judith McDonough, Cynthia Natiello, Randy Small, Marina S. Ferguson and Thomas S. Hatsukami

Circulation. 2005;111:2768-2775; originally published online May 23, 2005; doi: 10.1161/CIRCULATIONAHA.104.504167
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/111/21/2768