Characterization of Complicated Carotid Plaque With Magnetic Resonance Direct Thrombus Imaging in Patients With Cerebral Ischemia

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Background—Thromboembolic disease secondary to complicated carotid atherosclerotic plaque is a major cause of cerebral ischemia. Clinical management relies on the detection of significant (>70%) carotid stenosis. A large proportion of patients suffer irreversible cerebral ischemia as a result of lesser degrees of stenosis. Diagnostic techniques that can identify nonstenotic high-risk plaque would therefore be beneficial. High-risk plaque is defined histologically if it contains hemorrhage/thrombus. Magnetic resonance direct thrombus imaging (MRDTI) is capable of detecting methemoglobin within intraplaque hemorrhage. We assessed this as a marker of complicated plaque and compared its accuracy with histological examination of surgical endarterectomy specimens.

Methods and Results—Sixty-three patients underwent successful MRDTI and endarterectomy with histological examination. Of these, 44 were histologically defined as complicated (type VI plaque). MRDTI demonstrated 3 false-positive and 7 false-negative results, giving a sensitivity and specificity of 84%, negative predictive value of 70%, and positive predictive value of 93%. The interobserver (κ=0.75) and intraobserver (κ=0.9) agreement for reading MRDTI scans was good.

Conclusions—MRDTI of the carotid vessels in patients with cerebral ischemia is an accurate means of identifying histologically confirmed complicated plaque. The high contrast generated by short T1 species within the plaque allows for ease of interpretation, making this technique highly applicable in the research and clinical setting for the investigation of carotid atherosclerotic disease. (Circulation. 2003;107:3047-3052.)

Key Words: thrombus ▪ plaque ▪ carotid arteries ▪ imaging ▪ cerebral ischemia

Atherosclerotic disease is a major cause of cerebral ischemia. Embolization from the surface of an atherosclerotic plaque to cerebral vessels can result in occlusion, which, if sufficiently prolonged, will result in cerebral infarction. More transient vascular occlusion may result in temporary ischemia, producing a neurological deficit that recovers with little or no residual brain damage. Clinically, transient ischemic attacks (TIAs) provide a warning of future cerebral ischemic events; ≈10% of patients who sustain a TIA, left untreated, will suffer a definitive stroke in the following year,1 followed by a rate of 5% per annum. A warning TIA therefore offers the chance to intervene to prevent future permanent cerebral damage. The North American2 and European3 endarterectomy trials have both shown the positive benefit of surgery in patients with significant stenosis. The trials also indicate that lesser degrees of carotid disease are responsible for a significant number of strokes, but the risks of surgery matched or outweighed the benefits. Techniques have been sought to further define those among the group with moderate stenosis who are at high risk.

In 1995, Stary et al,4 for the American Heart Association, defined different atherosclerotic subtypes, the purpose being to pathologically identify plaque more likely to cause symptoms. Complicated plaque was defined as that with surface rupture or intraplaque or associated intraluminal hemorrhage and was defined as type VI disease. The presence of these features is associated with increased risk of thromboembolism and subsequent symptoms. In addition, it has been shown that the stepwise progression of atheroma, either with or without symptoms, in its latter accelerated phase is often associated with intraplaque hemorrhage, thus providing further evidence of the importance of this form of complex plaque in atherosclerotic disease.5 A means of identifying type VI complicated plaque in vivo may therefore provide a...
surrogate marker of plaque activity and an improved method of patient stratification and management.

MRI has the ability to discriminate between the different stages of thrombus and hemorrhage formation. In the acute/subacute phase, formation of methemoglobin results in shortening of $T_1$, producing an increase in signal on a $T_1$-weighted sequence. We have used such a sequence to detect acute/subacute thrombus in a number of clinical settings, including lower limb deep vein thrombosis and pulmonary embolus. In addition, we have also applied this technique in the setting of acute cerebral infarction for the detection of intraplaque hemorrhage. Imaging of the carotid bifurcations of patients who had suffered an acute (<24 hours) stroke demonstrated that 69% had high signal material within the carotid vessel wall ipsilateral to the hemisphere of their cerebral infarct. Of these, 45% had a stenosis >50%. This therefore suggested an association between the presence of high-signal material, presumed to be methemoglobin from recent plaque hemorrhage (ie, complicated plaque), and cerebral infarction, even in the absence of significant stenosis. To assess this further, direct comparison between the imaging findings and the pathophysiological process within the carotid vessel is required.

A means of making this comparison is provided by the histological examination of endarterectomy specimens after in vivo magnetic resonance direct thrombus imaging (MRDTI). The aim of this study, therefore, was to assess the accuracy of MRDTI in identifying carotid complicated plaque in vivo in patients suffering anterior cerebral circulation ischemia using histopathology as the "gold standard." In addition, the interobserver agreement for the detection of carotid high signal using MRDTI was assessed.

Methods
Ethics committee approval and informed consent were obtained for all patients entered into the trial. Between May 1998 and September 2001, 142 patients who were being assessed before carotid surgery were considered for scanning. All were known to have significant carotid stenosis (>70%) shown by ultrasound. Patients were recruited sequentially as dictated by availability of scanner time, which was just over 5.5 minutes long. In the latter part of the study, no delay time of 1000 ms that was used. The resulting scan acquisition took into consideration the incomplete relaxation afforded by the delay time of 1000 ms that was used. The resulting scan acquisition was just over 5.5 minutes long. In the latter part of the study, no delay time was used, reducing the scan time to 3.5 minutes with no detrimental effects on image quality of the plaques.

The 3D MRI data set was viewed on the scanner workstation by use of a multilayer reconstruction package. A complicated plaque was diagnosed if high-signal material was seen arising from the wall of the carotid artery anywhere in the region of and 1 cm on either side of the stenosis. High signal was defined as signal brighter than that arising from the adjacent muscle.

After surgery, the carotid endarterectomy specimens were collected in 10% formal saline. After fixation, the specimens were sectioned into blocks. The endarterectomy specimens were cut into 5-mm blocks, starting from the specimen base (the proximal or common carotid end) and progressing distally toward the bifurcation and beyond, until the whole length of the specimen had been cut. Specimens were processed routinely for paraffin embedding. The formalin-fixed, paraffin-embedded tissue blocks were serially sectioned at 4 μm onto slides and stored at room temperature until viewed. The first section of each block was stained with Gill’s hematoxylin and eosin 1%.

The specimen was viewed at ×4 to ×20 magnification, and complicated (type VI) plaque was diagnosed if any of the following features were observed in any of the specimens: free red blood cells within the intima or media not associated with the blood vessel lumen; organized lamellar plaque or luminal adherent thrombosis (lines of Zahn, platelets, fibrin, red blood cells, and white blood cells); hemosiderin-containing macrophages; or surface defects or rupture. Specimens were defined as either complicated (type VI) or noncomplicated.

Images judged to be nondiagnostic because of patient motion or insufficient removal of fat signal were not included. Histopathological specimens that were grossly fragmented were excluded. The sensitivity, specificity, and positive and negative predictive values were calculated from the results of one reader (A.R.M.) experienced in interpreting the MRDTI scans. The histology was used as the gold standard. A single (highest) rating was used for each technique, and no attempt was made to match MRI and histology on a slice-by-slice basis.

Intraobserver and interobserver variability were calculated for the first 40 patients. Intraobserver calculation compared the results with those of a second reader (R.E.M.) trained specifically for this project. Intraobserver and interobserver agreement was calculated by use of $\kappa$ statistics. All interpretations of the histological or MRI data were performed in a blinded manner. Calculations were performed by use of the Statistical Package for Social Sciences software (SPSS).

Results
Sixty-three patients, each with interpretable MRDTI and histological specimens for comparison, were recruited (Figure 1). Of these, 30 were women. The average age was 69 years (range, 47 to 86 years). Thirty-one (49%) had suffered
TIA alone, 7 (11%) amaurosis fugax alone, 6 (10%) both TIA and amaurosis fugax, 2 (3%) had had retinal artery occlusions, and 17 (27%) had recovered from anterior circulation strokes. The median time from the onset of first symptom to scanning was 12 weeks (interquartile range, 8 to 20 weeks).

Of the 63 specimens, 44 plaques were defined histologically as complicated (type VI) and 19 as noncomplicated (Figure 2). MRDTI demonstrated 16 true-negative (noncomplicated) lesions (Figure 3) and 37 true-positive (complicated) lesions (Figures 4 and 5), with 3 false-positive and 7 false-negative results. Of the 7 false-negative results, 2 had cap rupture/fissuring alone, 3 samples were slightly fragmented, and 2 had luminal thrombus alone. The sensitivity and specificity for MRDTI detecting complicated plaque was 84%, with a positive predictive value of 93% and negative predictive value of 70%. The $\kappa$ statistics for interobserver and intraobserver agreement for MRDTI were 0.75 and 0.9, respectively (substantial agreement).

Discussion

Summary of Findings

The aim of this study was to assess the accuracy of MRDTI in identifying carotid complicated plaque in vivo in patients with anterior cerebral circulation ischemia using histopathology as the gold standard. We have shown that MRDTI has relatively high sensitivity (84%) and specificity (84%) for identifying complicated plaque; high signal on MRDTI had a positive predictive value of 93% for complicated plaque. In addition, the interobserver and intraobserver agreements for MRDTI were 0.75 and 0.9, respectively (substantial agreement).

Discussion of Findings

This is the first in vivo human study of carotid atheromatous disease in a symptomatic population aimed specifically at detecting complicated plaque by detecting intraplaque hemorrhage by MRI. On the basis of the hypothesis that complicated plaque is commonly associated with histological evidence of plaque hemorrhage or thrombosis, we used an MRI technique that exploits the $T_1$-shortening properties of recent thrombus to identify carotid thrombus and thus complicated plaque. Application of a similar technique elsewhere in the vascular system has shown the rapid formation of the $T_1$-shortening species in thrombus (within 8 hours) and its persistence for a number of weeks once formed.

The high prevalence (40/63, or 64%) of MRDTI-defined complicated plaque in this selected symptomatic group supports the correlation between its presence and symptoms. This rate of detection is similar to that seen in our previous work, in which 69% of acute stroke patients demonstrated ipsilateral carotid high signal on MRI scanning. A larger study of prevalence in symptomatic and asymptomatic arteries is needed to investigate this further. The longevity of intraplaque signal is not known and also requires further study before this technique can be used as a marker of recent plaque disruption. However, if the MRI high signal can be shown to be limited to a finite time span, its presence could be used to accurately identify recent plaque thrombosis or hemorrhage.

It is recognized that these events may occur in the absence of stenosis, but no information regarding the relationship between stenosis and MRI high signal was obtained from this study, because all recruited patients had, by trial entry criteria, >70% stenosis. By detecting complicated plaque noninvasively, MRDTI offers the attractive potential of...
studying both the natural history of plaque and its response to interventions as well as identifying asymptomatic disease and symptomatic disease in nonstenotic vessels.

The ease of scan interpretation is reflected in the high interobserver agreement. Detection of complicated plaque is therefore relatively straightforward, allowing application in the research and clinical setting. Alternative imaging strategies require the measurement of stenosis or differentiation of plaque contents to characterize carotid disease, all of which are operator dependent; studies of interobserver variability using these criteria have shown significant variation. We have not attempted to assess the extent of intraplaque high signal and its relationship to other plaque constituents such as cholesterol pool, cap thickness, or neovessels, although the interplay between each of these may have a significant role in symptom development and plaque progression.

**Strengths of the Present Study**

This technique is easy to apply on most commercially available scanners, requiring no additional hardware or software. Image acquisition is not operator dependent except for the routine scan setup. Like other MRI techniques, it is noninvasive, requires no ionizing radiation, and is quick to perform (3.5 minutes). All these attributes led to improved
patient acceptability and ease of performing follow-up studies. Being a 3D acquisition, reconstruction allows multiplanar visualization of vessel-wall disease and the potential for segmentation of hemorrhage volume. As with other black-blood techniques, removal of high blood signal diminishes flow artifacts that may cause image degradation. Because disease detection does not depend on stenosis, disease overestimation by artificial signal loss seen with magnetic resonance angiography of flowing blood is not an issue. The use of a water excitation pulse to remove fat signal has recently been shown to be faster and to produce improved image quality in comparison with other fat-removing techniques.\(^{13}\)

**Limitations of the Present Study**

This technique is reliant on the removal of high fat signal normally seen on T\(_1\)-weighted images. Failure of the water excitation RF pulse to achieve this results in the high-signal complex plaque being undetectable. We found that manual shimming of the magnet at the beginning of the imaging procedure resulted in the most consistent diagnostic images. Although the overall scan acquisition time was short, movement, especially swallowing, could lead to nondiagnostic scans because of motion artifacts, and this accounted for most of the cases of uninterpretable MRI scans. Some of the differences between MRI and pathology resulted from using the broader histologically defined complicated (type VI) plaque as the gold standard. A number of different features that are invisible to the MRI technique described here were used to define complicated plaque histologically. These included the presence of hemosiderin-laden macrophages and the presence of fibrous cap fissuring alone. In addition, the injection of intraluminal thrombus, with fewer trapped red blood cells, may have different signal characteristics compared with a hemoglobin-rich intraplaque hemorrhage.\(^{14}\) Despite these differences, we wished to test the usefulness of this technique in a clinical setting, which required all types of uninterpretable MRI scans. Some of the noncomplicated subtypes.\(^{23,24}\) Recent animal experiments have also shown the ability of MRI to accurately identify and age intraluminal\(^{14}\) thrombus and that arising from induced thrombosis.\(^{25}\)

**Implications and Future**

In the future, dedicated surface coils for the imaging of the carotid bifurcation will provide improved spatial resolution, allowing accurate correlation between the site of high signal within the MR image and the histological specimen. An alternative approach would be to re-image the endarterectomy specimens in vitro at higher field strength and for prolonged imaging times to produce high-resolution images. These images can then be “fitted” to the histological studies by computer manipulation to allow more accurate identification of the short-T\(_1\) species within the specimen. Combination with immunostaining for other constituents within the plaque, such as matrix metalloproteins, tissue factor, fibrin, macrophages, and circulating markers of atheromatous plaque activity,\(^{27}\) may also shed light on the pathophysiological process within the plaque. Accepting that this technique does identify complicated plaque, further study of these appearances and how they relate to the natural history of vessel wall disease is needed. Importantly, this will determine the lifespan of the high signal and its relationship to plaque progression and symptoms. The results of this study should define the clinical applications of this technique, its role as a marker of vulnerable plaque, and its use as a surrogate marker of disease progression or regression in clinical or therapeutic trials.

**Conclusions**

In conclusion, 3D T\(_1\)-weighted imaging of the carotid vessels in patients with cerebral ischemia is an accurate means of identifying histologically confirmed complicated plaque. The high contrast generated by short-T\(_1\) species within the plaque allows for ease of interpretation, making this technique highly applicable in the research and clinical setting for the investigation of carotid atherosclerotic disease.

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