Quantitative Magnetic Resonance Imaging Analysis of Neovasculature Volume in Carotid Atherosclerotic Plaque

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Background—Neovasculature within atherosclerotic plaques is believed to be associated with infiltration of inflammatory cells and plaque destabilization. The aim of the present investigation was to determine whether the amount of neovasculature present in advanced carotid plaques can be noninvasively measured by dynamic, contrast-enhanced MRI.

Methods and Results—A total of 20 consecutive patients scheduled for carotid endarterectomy were recruited to participate in an MRI study. Images were obtained at 15-second intervals, and a gadolinium contrast agent was injected coincident with the second of 10 images in the sequence. The resulting image intensity within the plaque was tracked over time, and a kinetic model was used to estimate the fractional blood volume. For validation, matched sections from subsequent endarterectomy were stained with ULEX and CD-31 antibody to highlight microvessels. Finally, all microvessels within the matched sections were identified, and their total area was computed as a fraction of the plaque area. Results were obtained from 16 participants, which showed fractional blood volumes ranging from 2% to 41%. These levels were significantly higher than the histological measurements of fractional vascular area. Nevertheless, the 2 measurements were highly correlated, with a correlation coefficient of 0.80 ($P < 0.001$).

Conclusions—Dynamic contrast-enhanced MRI provides an indication of the extent of neovasculature within carotid atherosclerotic plaque. MRI therefore provides a means for prospectively studying the link between neovasculature and plaque vulnerability. (Circulation. 2003;107:851-856.)

Key Words: magnetic resonance imaging ■ contrast media ■ carotid arteries ■ atherosclerosis

Recent investigations have targeted neovasculature as an important factor contributing to atherosclerotic plaque vulnerability. Zamir and Silver1 speculated that the presence of neovasculature within coronary artery walls may play a role in the pathogenesis of vascular disease. Kumamoto et al2 showed that intimal neovasculature arising from the adventitial vasa vasorum was associated with inflammatory infiltrate. A role for neovasculature in the recruitment of leukocytes to the shoulder regions of lipid-rich plaques was proposed by de Boer et al.3 Because such inflammatory cells are present at the sites of plaque rupture,4 neovasculature may be a contributor to or a marker for vulnerable plaque. Support for this claim is provided by Mofidi et al,5 who found higher microvessel counts in carotid endarterectomy specimens from symptomatic patients than from asymptomatic ones. Similarly, McCarthy et al6 found microvessels in symptomatic patients were larger and more irregular in shape.

These studies suggest that an imaging tool capable of measuring the extent of plaque neovasculature could be invaluable for identifying high-risk plaques or assessing the response to plaque-stabilizing therapies. A strong contender for measuring plaque neovasculature is MRI using an intravenously injected contrast-enhancing agent. Contrast-enhanced (CE) MRI has been used in numerous studies to investigate the vascularity of tumors.7–9 In the study of atherosclerosis, CE-MRI performed on pigs showed an enhancing outer rim surrounding the walls of carotid arteries 4 weeks after balloon injury.10 Aoki et al11 showed similar enhancement of the outer rims of human carotid arteries. The cause of enhancement is thought to be increased vascularity of the adventitial vasa vasorum feeding the plaque neovasculature. Yuan et al12 and Wasserman et al13 observed enhancement of the plaque interior that was related to fibrous tissue. In the former study, evidence of hyperenhancement related to especially dense and large neovasculature was also found. Although these studies suggest a link between enhancement patterns in CE-MRI and neovascularity, they do not provide a means for quantitative comparisons of the number or size of plaque microvessels. The ability to make quantitative comparisons of neovasculature must be estab...
lished to use CE-MRI in investigations of neovasculature and its impact on plaque vulnerability.

The purpose of the present study was to investigate whether dynamic CE-MRI can provide a quantitative measurement that reflects neovasculature in carotid atherosclerotic plaques immediately before endarterectomy. High-resolution, dynamic CE-MRI sequences were obtained and analyzed using a kinetic model of contrast agent dynamics. The model included a parameter for fractional blood volume (fBV), and the average value of fBV within the plaque was taken as the neovasularity of the plaque. To test this parameter as an indicator of the extent of neovasculature, it was compared with the fractional area of microvessels in corresponding histological sections of the endarterectomy specimens.

**Methods**

**Magnetic Resonance Imaging**

Between March 2000 and March 2002, a total of 20 consecutive patients scheduled for carotid endarterectomy were recruited for an MRI examination including a dynamic CE-MRI protocol. Informed consent was obtained from all patients, and the study was approved by the institutional review board at the University of Washington. The MRI examination was conducted within 1 week before endarterectomy, wherein the plaque was removed intact for histological analysis. All patients were imaged on a 1.5T MRI scanner (Signa Horizon EchoSpeed 5.8, GE Medical Systems) using specially-designed, phased-array surface coils. The examination included sequences of axial 2D spoiled gradient-recalled echo, T1-weighted images of the diseased carotid artery at 5 to 7 locations, centered on the carotid bifurcation. The imaging parameters were as follows: repetition time (TR), 100 ms; echo time (TE), 3.5 ms; flip, 60°; thickness, 3 mm; gap, 1 mm; field-of-view, 16×12 cm; and matrix, 256×144. Each acquisition was repeated 10 times, with a repetition interval of 15 seconds. Coincident with the second image in the sequence, 0.1 mmol/kg (0.2 mL/kg) gadolinium-based contrast agent (Omniscan, Amersham Health) was injected at a rate of 2 mL/s via a power injector.

After acquisition, one location per patient was selected for analysis. The primary criteria for selection were the presence of a large atherosclerotic plaque and clear delineation of the lumen and outer wall boundaries of the artery. If more than one location met these criteria, the one closest to the bifurcation was selected to facilitate matching with histological specimens.

Each selected image sequence was then individually post-processed with the Kalman Filtering Registration and Smoothing (KFRS) algorithm, which reduced patient motion and noise in the image sequences. Finally, contours were drawn around the carotid lumen and just inside the outer wall boundary using the first image after contrast agent arrival in the carotid lumen. The outer contour was carefully drawn to exclude the adventitia, thereby limiting the analysis region to the plaque itself. Contrast agent kinetics were analyzed for the plaque region between contours.

**Kinetic Modeling**

To analyze contrast agent dynamics, an approach similar to one used by Roberts et al was used to measure fBV. This approach assumes that (1) contrast agent concentration in the plaque consists of a blood component (BBV) and an extravascular extracellular space (EES) component, (2) contrast agent concentration is proportional to the change in signal intensity, (3) plasma concentration decays exponentially, and (4) reflux of contrast agent from the plaque to the plasma can be neglected. The assumption that concentration is proportional to signal intensity change is approximately correct for sufficiently low concentrations of contrast agent and permits the intensity change to be used in lieu of concentration. Neglecting contrast agent reflux is the central feature of the model of Patlak et al and is appropriate for studies of short duration.

Given these assumptions, the plasma concentration will be: 
\[ C_p(t) = A e^{-m t}, \]
where \( C_p(t) \) is the plasma concentration (ie, intensity change) at time \( t \), \( A \) is the initial concentration, and \( m \) is the rate of decay. The tissue concentration will be: 
\[ C_t(t) = fBV C_p(t) + k C_p(u) du, \]
where \( C_t(t) \) is the tissue concentration, \( k \) is a rate parameter for the transfer of contrast agent from plasma to EES, \( u \) is a variable of integration, and the integral is evaluated from 0 to \( t \).

The parameters in the blood and tissue concentration models were computed from the image sequences. \( A \) and \( m \) were found by averaging the change in signal intensity from 8 to 10 pixels within the jugular vein and fitting an exponential by minimizing the sum of squared differences. The jugular vein was chosen because it was the nearest large vessel exhibiting the blood signal (stenosis of the carotid artery prohibited use of the artery itself). The resulting plasma curve was then used to evaluate the expression of \( C_p(t) \). The parameters fBV and \( k \) were found individually for every pixel in the plaque by linear regression using \( C_t(t)/C_p(t) \) as the dependent variable and \( \int C_t(u) du/C_p(t) \) as the independent variable. The average value of fBV over the entire plaque was then recorded.

**Histological Validation**

To assess the ability of the kinetic model to quantify neovasculature, the endarterectomy specimens were histologically examined. Sections 10 μm thick were obtained every 0.5 mm in the vicinity of the bifurcation and stained with hematoxylin and eosin. The best matching section was identified for each image analyzed before performing the kinetic analysis. The best match was based on position relative to the bifurcation and overall shape of the lumen and plaque. Additional sections from the same location were then stained using ULEX and CD-31 antibody, which highlight endothelial cells lining the neovasculature.

After staining, the sections were photographed and scanned into a computer at high resolution. A custom program for semiautomated identification of microvessels was then used to measure the total area lying within the microvessels. This measurement was divided by the

**Figure 1.** Dynamic contrast-enhanced MRI. a, Precontrast image with approximate lumen and plaque outer boundaries indicated. b, Image acquired at initial arrival of contrast agent in the jugular vein (J) shows foci of enhancement (arrow). c, Image acquired 45 s later shows increasingly diffuse enhancement. d, Diffuse enhancement continues at 90 s after contrast agent arrival.
total plaque area and recorded as the fractional vessel area (fVA). The fVA was used as the “gold standard” for plaque neovascularity. The comparison between the fVA by histology and the average fBV by MRI was made by computing the correlation coefficient (R) and its associated statistical significance (P).

Results

Of the 20 original patients, 4 were subsequently deleted from the study as a result of (1) damage to the endarterectomy specimen precluding histological analysis, (2) excessive motion blurring of the images, (3) image prescription that missed the plaque location, and (4) a circuitous vessel course that led to partial volume averaging artifacts (1 patient each). Imaging results from one of the 16 remaining patients are shown in Figure 1, and corresponding kinetic modeling results are shown in Figure 2. Figure 2 also demonstrates how the inner and outer boundaries of the plaque were drawn and how pixels were selected from the jugular vein to generate the blood curve. The time versus intensity plot shows consider-

Figure 2. Model fitting (a) image at bolus arrival showing the inner and outer boundaries of the plaque, pixels within the jugular vein (+), a pixel on the outer rim of the carotid artery (●), a slowly enhancing pixel (▲), and a rapidly enhancing pixel (●). b, The average signal increase versus time for the pixels in the jugular vein and for the 3 selected pixels within the artery wall. The solid lines are the model fit to the data. Note that the diamond appears outside the plaque boundary because the adventitia was excluded from the analysis of plaque enhancement.

Figure 3. Histology corresponding to Figure 2: a, section stained with CD-31 antibody; b, magnified view of region indicated in panel a; and c, same region with microvessels identified.
ably more enhancement for the pixel marked with the circle than the pixel marked with the triangle. Corresponding histology, shown in Figure 3, shows the circle is located in an area of dense neovasculature, whereas the triangle is in a region devoid of neovasculature. This patient had the highest fVA by histology (2.6%) and the second highest average fBV (19.7%).

Figure 4 shows a parametric image of fBV for another highly vascular plaque. The lumen of the internal carotid artery, external carotid artery, and jugular vein appear bright, indicating 100% blood volume. A large blood volume is also observed in a region of hemorrhage in the center of the plaque. This region was fed by numerous microvessels that are visible in histology. Also of note is a moderately large blood volume forming a ring around the outer rim of the vessel wall. This outer rim was apparent in all plaques, but was excluded from the analysis by drawing the outer boundary such that only the internal plaque was included.

Finally, a plot of average fBV versus neovascularity for all patients is shown in Figure 5. A trend of higher fBV measurements with greater neovascularity is evident. The correlation coefficient for the 2 measurements is 0.80 (P<0.001). Regression analysis yielded a trend line with an intercept of 3.1 and a slope of 7.7.

Discussion
Because neovasculature has been implicated in plaque progression, instability, and occurrence of ischemic symptoms, a noninvasive method for measuring the degree of plaque neovascularity could provide a marker for atherosclerotic plaques with increased risk of rupture. Quantitative measurements of neovasculature could then be used to identify patients in need of aggressive intervention, such as endarterectomy, or to monitor plaques over time for signs of progression or stabilization. This study demonstrates the potential value of dynamic CE-MRI for such quantitative analysis. Specifically, dynamic CE-MRI was shown to correlate strongly with the fractional area of neovasculature, which captures both the density of microvessels in number and size. Both of these factors have been tied to a history of symptoms. Dynamic CE-MRI could thus be used to investigate whether an apparent link exists between neovasculature and future symptoms.

One interesting aspect of this study was that for 3 of the specimens, the histological measurement of fVA was >1.5%, which is considerably higher than the remaining specimens. As shown in Figure 5, these 3 specimens also had the highest fBV by MRI, indicating that this technique is suitable for detecting such highly vascular plaques and investigating their role in disease progression.

These results add further merit to CE-MRI study of atherosclerosis. In previous investigations, post-contrast enhancement was observed on black-blood, T1-weighted images in fibrous regions, suggesting that CE-MRI is valuable for assessing the state of the fibrous cap, another important factor in plaque rupture. The methods used in these studies may not, however, be appropriate for investigating neovasculature because the images were obtained several minutes after injection at a time when significant amounts of contrast agent had been transferred from the vasculature to the EES. Also, the use of black blood preparation (eg, double inversion recovery) for best separation of the fibrous cap from the lumen may lead to suppression of the signal from the neovasculature. To enable visualization of enhancement in both fibrous and neovasculature-rich regions, a combination of an initial dynamic, bright-blood CE-MRI sequence with a later, post-contrast, black-blood CE-MRI sequence is recommended.

In this study, no attempt was made to localize regions of neovasculature within the cross-sectional images. Location may be an important factor because focal neovasculature in the shoulder regions of the plaque is of greater concern than

![Figure 4. Parametric map. a, Histology specimen (Mallory's trichrome stain) showing a small lumen (L), a large region of hemorrhage (long arrow) at the center of the plaque, and numerous small microvessels throughout the plaque. b, Map of fBV where the vessel lumen (L) appears bright, indicating 100% blood volume, the area of hemorrhage (long arrow) shows a large blood volume, and the outer rim of the vessel (short arrow) shows a moderate blood volume. Black regions contain very little blood volume.](image)

![Figure 5. Comparison of fBV (in percent) by MRI and fVA (in percent) by histology.](image)
diffuse neovascularure. Unfortunately, the average fBV measured here tends to produce lower measurements for focal neovascularure because avascular regions reduce the average response. This phenomenon seems to be responsible for some of the spread in the data. The image sequences do, however, show potential for distinguishing regional differences in neovascularure, as evident in Figures 2 and 4. Spatial variation was not investigated in this study because of the limited spatial resolution of the dynamic sequence used. Improving the resolution of the imaging sequence is under investigation.

The analysis presented here used the assumption of Patlak et al. that the contrast agent only moves into the plasma, not subsequently back into the plasma. Although this assumption is almost certainly untrue (as evidenced by the dips in intensity over time apparent in Figure 2), this model was the best option among commonly used models. Assuming that backflow existed, on the other hand, led to a model that was unstable in regions dominated by large blood volumes. This instability arose because a strong blood-like signal could be modeled either as a large fBV or as a large EES with a very large rate of contrast agent transfer. Another common model used for analyzing dynamic CE-MRI presented by Tofts and Kermode assumes that the blood volume is negligible. Thus, fBV can only be inferred from other model parameters. Identifying a model that optimally captures contrast agent dynamics in atherosclerotic plaque is a subject for future research.

Future investigation will also seek to use the rate of enhancement in addition to the fBV. Differences in enhancement rate may indicate microvessels of different sizes or distinguish neovascularure from EES. Slowly enhancing regions, in particular, are likely to indicate areas with considerable amounts of EES, such as loose matrix.

The most significant issue with the current analysis is that the average fBV values are considerably higher than the fVA values from histology. The strong correlation between measurements suggests that fBV from MRI can be used as an indicator of neovascularure. However, an understanding of the factors that may be contributing to the overestimation is important for interpretation of the results. Some variability may arise because the images are 3 mm thick and the histology sections are 10 μm thick. This should not have an effect, however, if the fVA remains approximately constant throughout the 3-mm image thickness, in which case true measurements of fVA and fBV would be the same. Some variability may, nevertheless, arise because of regional variations in the rate of blood flow. Another contribution to the discrepancy is that assuming no contrast agent reflux will lead to overestimation of the fBV if reflux occurs. As mentioned above, some reflux is apparent. A third factor is that some distortion and shrinkage of the specimen takes place in histological processing that may reduce the apparent vascular area. Because no collapse of vessels was observed, this effect is thought to be minor.

The largest contribution to the overestimation of neovascularure volume is likely due to interstitial volumes undergoing very rapid exchange of the contrast agent with the blood plasma. Any such regions that come to equilibrium within one time frame of the dynamic sequence will be indistinguishable from blood and therefore included in fBV. The contrast agent we used has a low molecular weight and rapidly distributes into the extracellular fluid, making this the most likely cause of overestimation. Evidence that such rapid exchange contributes to the overestimation is provided by the observation that areas of recent hemorrhage, such as in Figure 4, often exhibited the highest fBV. These recent hemorrhages are likely to have high permeability. Such volumes of rapid exchange could, perhaps, be distinguished if the temporal resolution was increased to a few seconds or less. Alternatively, the use of contrast agents that remain within the blood pool or diffuse more slowly might further separate intravascular space and EES. Such agents are currently being developed.

The contribution of the EES to the apparent fBV may also be amplified by our assumption of a linear relationship between concentration and signal change. In reality, increasing concentration yields progressively smaller changes in signal intensity. Thus, a region with moderate concentration will appear overly large when normalized using a high concentration region such as the blood. Overcoming this nonlinearity requires maps of the T1 values of the tissue, which has not yet been achieved in vivo for plaque imaging.

Further investigation is needed to determine if the factors leading to overestimation of fBV can be eliminated. Despite the apparent overestimation, the correlation remained strong, making quantitative comparisons possible using the current methods and temporal resolution. This technique could therefore be used in a prospective study of neovascularure. If subjects who developed stroke or related symptoms had higher initial values of fBV by MRI, this would suggest that neovascularure plays a role in plaque rupture. Similarly, if this technique is used in serial studies, changes in the measured fBV would likely indicate corresponding changes in plaque neovascularure.

In conclusion, kinetic modeling of dynamic CE-MRI to measure fBV is a quantitative technique that reflects the neovascularity of atherosclerotic plaques. Because increased density and size of plaque microvessels are thought to be associated with increased risk, this approach has great promise for identifying vulnerable plaques in vivo. Further study is warranted to determine the predictive power of dynamic CE-MRI for identifying patients at risk for plaque progression or developing symptoms and to determine if CE-MRI can be used to characterize inflammation and loose matrix components of plaque.

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References
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