Cerebrovascular disease is the third most common cause of death and one of the leading causes of disability.1,2 Carotid artery atherosclerosis is a known risk factor for the development of stroke.3 Multiple randomized multicenter controlled trials have evaluated the benefit of carotid endarterectomy (CEA) compared with medical treatment. These trials have stratified the risk of stroke for symptomatic and asymptomatic patients with various degrees of luminal stenosis. The North American Symptomatic Carotid Endarterectomy Trial (NASCET)4 and European Carotid Surgery Trial (ECST)5 reported significant benefit of CEA versus medical treatment in symptomatic patients with severe (>70%) carotid stenosis, whereas in patients with 50% to 70% stenosis, the benefit was modest.3 The Asymptomatic Carotid Atherosclerosis Study (ACAS)6 and Asymptomatic Carotid Surgery Trial (ACST)7 demonstrated the beneficial effect of CEA for asymptomatic patients with severe carotid stenosis and low perioperative risks. However, the benefit of CEA is less clear in asymptomatic patients with 50% to 70% internal carotid artery (ICA) stenosis. Although the observations in the ACST were significant, 40 CEAs were required to prevent 1 fatal or disabling stroke.8 Thus, identification of specific characteristic features that allow further stratification of patients at risk of stroke remains a matter of critical clinical importance. Currently, in addition to stenosis severity, much attention is focused on the identification of the vulnerable plaque. This term is used to describe atherosclerotic plaques with a high risk of causing neurological symptoms. Noninvasive imaging can help to identify specific characteristics of the atherosclerotic plaque that are associated with an increased risk of stroke.9 Plaque neovascularization and adventitial vasa vasorum (VV) play an important role in the pathogenesis of the vulnerable plaque.10–12

Vasa Vasorum Enhancement on Computerized Tomographic Angiography Correlates With Symptomatic Patients With 50% to 70% Carotid Artery Stenosis

Javier M. Romero, MD; Raffaella Pizzolato, MD; Wendy Atkinson, BA; Anna Meader, BSc; Camilo Jaimies, MD; Glenn Lamuraglia, MD; Michael R. Jaff, DO; Ferdinando Buonanno, MD; Josser Delgado Almandoz, MD; Ramon G. Gonzalez, MD, PhD

Background and Purpose—Significant stenosis of the internal carotid artery (ICA) is an established stroke risk factor. Recent evidence suggests that features within the atherosclerotic plaque also have prognostic value. The purpose of this study was to correlate the enhancement of the vasa vasorum (VV) overlying the carotid artery plaque with acute neurological symptoms in patients with 50% to 70% ICA stenosis.

Methods—We conducted a 4-year retrospective computerized tomographic angiographic review to identify patients with 50% to 70% stenosis of the ICA. Three types of plaques were identified: enhancing VV, calcified, and none-enhancing-noncalcified. Medical records were reviewed for cardiovascular risk factors and neurological status, and imaging was reviewed for signs of a recent stroke.

Results—We identified a total of 428 patients with 50% to 70% ICA stenosis: 103 (24.1%) had enhancing VV, 202 (47.2%) calcified, and 123 (28.7%) none-enhancing-noncalcified arteries; 97 were symptomatic and 331 asymptomatic. Thirty-three (34%) symptomatic subjects demonstrated enhancing VV, 42 (20%) had calcified arterial plaques, and 22 (17%) had none-enhancing-noncalcified arterial plaques. Fisher exact tests revealed that the proportion of symptomatic individuals with enhancing VV plaque was double that of the other groups combined (P=0.015; odds ratio, 1.92; 95% confidence interval, 1.17–3.16). Regression analyses confirmed this association as independent from other known cardiovascular risk factors.

Conclusions—In patients with 50% to 70% ICA stenosis, VV enhancement recognized on computed tomographic angiography is strongly associated with acute neurological symptoms compared with calcified and none-enhancing-noncalcified arterial plaques. This finding may aid in the identification of patients at increased risk for ischemic stroke within populations with the same degree of stenosis. (Stroke. 2013;44:3344-3349.)

Key Words: atherosclerosis ◼ carotid artery diseases
VV overlying carotid plaque can be appreciated on different imaging modalities and has been linked to increased risk of vascular events.\textsuperscript{13-16} Several studies have demonstrated the correlation of carotid wall enhancement on micro-computerized tomography (CT) and MRI with VV neovascularization in histological specimens.\textsuperscript{13,17} In patients with \textgeq 70\% carotid stenosis, enhancement of the VV on CT angiography (CTA) is associated with acute neurological symptoms.\textsuperscript{24} The purpose of this study was to determine whether a significant relationship exists between acute neurological symptoms and enhancement of the VV in patients with moderate (50\%--70\%) ICA stenosis.

Materials and Methods

We obtained approval from our institutional review board to perform this study. A waiver of consent was granted. Research activities were in full accordance with all applicable federal and state regulations, including the Health Insurance Portability and Accountability Act privacy rule.

Patient Selection

Through our neuroimaging database, all neck CTAs performed at our institution during a 4-year period were selected; all patients with ICA atheromatous stenosis between 50\% and 70\% were included in our study. The strokes were classified according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification.\textsuperscript{19} Exclusion criteria included patients with technically suboptimal studies, bilateral moderate–severe carotid stenosis, and prior carotid revascularization (CEA, carotid artery stent). Patients with stroke derived from sources other than the ipsilateral ICA (target vessel), such as from cardiac embolism and lacunar strokes, were considered asymptomatic from the carotid artery.

Image Acquisition

Noncontrast CT and multidetector CTA acquisitions were performed according to standard protocols on either 16- or 64-section helical CT scanners (Light-Speed; GE Healthcare, Milwaukee, WI). Noncontrast CT examination was performed using an axial technique with 120 to 140 kV (peak), 170 mA, 2-second scanning time and 5-mm-section thickness reconstruction. Multidetector CTA was subsequently performed by scanning from the base of the C1 vertebral body to the vertex using an axial technique and the following parameters: pitch, 0.5; collimation, 1.25 mm; maximal milliampere, 350; kV (peak), 120; field of view, 22 cm; and 65 to 85 mL of nonionic contrast material administered by power injector at 4 to 5 mL per second into an antecubital vein. In all cases, Smart-Prep semiautomatic contrast bolus tracking technique was used; this technique triggered scanning after an opacification of 50 Hounsfield units (HU) in the ascending aorta was reached (GE Healthcare).

Imaging Analysis

Internal Carotid Artery Stenosis

The percentage of ICA stenosis identified in the radiology report was validated using the NASCET method on the CTA source images (CTA-SI).\textsuperscript{20}

Carotid Artery VV Detection

Two neuroradiologists (J.D.A., J.M.R.), with 7 and 15 years of experience, individually categorized each carotid plaque. During image review, a standardized center level of 200 HU and a window width of 750 to 760 HU were consistently used to allow optimal characterization of the plaque and avoid technical bias.\textsuperscript{21} Each radiologist described the appearance of the carotid plaque on CTA-SI as enhancing (Figure 1), calcified (Figure 2), or nonenhancing-noncalcified (Figure 3). A plaque was considered calcified if it measured \textgeq 130 HU and the hyperdense component involved \textgeq 50\% of the entire carotid plaque area at the maximum point of stenosis.\textsuperscript{22,23} A plaque was considered to be enhancing if \textgeq 50\% of the wall circumference enhanced at the level of maximum stenosis.\textsuperscript{18} Subsequent to visual assessment, region of interest (Analyze 6.0, Mayo Clinic, Rochester, MN) analysis included enhancing 50\% of the target ICA wall circumference and comparing it with that of the contralateral ICA wall. A minimum mean difference of 10 HU was considered meaningful and

Figure 1. A, Patient 1, axial computerized tomographic (CT) angiography of the neck demonstrates enhancement of the adventitial vasa vasorum of the proximal right internal carotid artery (black arrow). B, Axial diffusion MRI demonstrates restricted water diffusion of the right middle cerebral artery territory, representing an acute infarct. C, Patient 2, parasagittal CT angiography of the neck demonstrates enhancement of the adventitial vasa vasorum of the proximal left internal carotid artery (LICA; white arrow). D, Axial noncontrast CT of the head demonstrates a subacute infarction of the left upper parietal lobule.
was interpreted as enhancement. A plaque that did not meet the above parameters was considered as nonenhancing.

**Clinical Data**

Patient medical records were reviewed to retrieve information on acute neurological events and the presence of cardiovascular risk factors. A patient was considered symptomatic if an anterior circulation stroke or transient ischemic attack ipsilateral to the ICA stenosis was documented within 30 days of the CTA examination. The diagnosis of stroke was based on clinical data and either positive diffusion-weighted imaging or noncontrast CT findings at follow-up. Patients who had CTA for reasons other than stroke, who had vascular events outside the 30-day time frame, in a distribution not referable to the ICA or with infarcts not related to carotid artery disease (ie, multiple infarcts due to cardiogenic emboli) were considered asymptomatic from the ICA stenosis perspective.

Cardiovascular risk factors, including hypertension (defined by blood pressure ≥140/90 mm Hg or use of antihypertensive drugs), hypercholesterolemia (defined by low-density lipoprotein cholesterol >130 mg/dL), atrial fibrillation, coronary artery disease (confirmed by stress test or coronary angiography, history of myocardial infarction), diabetes mellitus (defined by the presence of fasting plasma glucose >126 mg/dL and hemoglobin A1c >6.4% or receiving medication for diabetes mellitus), and active and nonactive tobacco consumption, were gathered for these subjects and are summarized in Table 1.

**Statistical Analysis**

We used descriptive statistics and means of central tendency to summarize data on cardiovascular risk factors, stenosis of the ICA, symptoms, and appearance of the plaque. A Fisher exact test analysis was used to compare the frequency of symptomatic patients between the different types of plaques. Paired odds ratio (OR) analyses were used to estimate the strength of association between the types of plaque and the presence of symptoms. A logistic multivariate regression analysis was used to determine whether a significant association existed between the type of plaque and the development of acute neurological symptoms after controlling for cardiovascular risk factors and degree of stenosis. GraphPad Prism version 5.0c for Mac (GraphPad Software, San Diego, CA, http://www.graphpad.com) was used for Fisher exact test and χ2 test; MedCalc version 12.5.0 for Windows (MedCalc Software, Inc, Mariakerke, Belgium) was used to compute multivariate logistic regression analysis; agreement between the visual assessment and HU difference for VV detection was calculated using Cohen unweighted κ statistics.

**Results**

Eight thousand three hundred ninety neck CTAs were identified in the Massachusetts General Hospital neuroradiology database during the study period. Of these, 511 patients had an ICA stenosis of 50% to 70% (6%). Thirty-eight patients were excluded for stenosis secondary to acute or chronic carotid dissection. We excluded 24 patients with bilateral moderate carotid stenosis, 16 patients with previous revascularization procedures, and 5 patients with technically limited examinations.

Four hundred twenty-eight patients (243 men, 185 women; mean age, 73.3 years; SD, 10.4) with 50% to 70% ICA stenosis (right ICA=222; left ICA=206) fulfilled our inclusion criteria. No significant statistical difference was detected between male and female incidence and stenotic side affected (P=0.92; Fisher exact test). The mean percent diameter stenosis was 57.73% (SD=5.7). Data collected from clinical records and imaging identified 331 (77.3%) asymptomatic and 97 (22.7%) symptomatic patients (78 strokes, 19 transient ischemic attacks). No significant differences were noted on sex and ICA side with stroke symptoms (P=0.82 and P=0.06

**Figure 2. A and B** Axial computerized tomographic angiography demonstrates a dense calcified plaque (black arrows) at the origin of the left internal carotid artery.

**Figure 3. A and B** Axial computerized tomographic angiography of the neck demonstrates nonenhancing-noncalcified plaque (black arrows) of the proximal left internal carotid artery.
Two cases that were interpreted visually as enhancing VV did not enhance on the HU measurements, and 1 case that apparently did not enhance visually did enhance on the HU measurements. Complete agreement was noted on the assessment of calcified plaques (κ=0.98 [95% CI, 0.96–1]). There was a near-perfect agreement between the visual assessment and the HU measurement method. Interobserver visual agreement was excellent (κ=0.80).

Discussion

ICA stenosis is a recognized risk factor for stroke, and the identification of vulnerable plaque features is important because such characteristics could be used as predictors of stroke and transient ischemic attack in patients with the same severity of ICA stenosis. This study aimed to correlate the enhancement of the VV overlying the carotid artery plaque with acute neurological symptoms in patients with moderate 50% to 70% stenosis. Interestingly, our study demonstrated that ICAs with enhancing VV are more likely to be symptomatic than those that are calcified or nonenhancing-noncalcified in the carotid wall. The enhancing group demonstrated a doubling in the risk of symptoms compared with the calcified and nonenhancing-noncalcified groups (OR=1.80 and OR=2.16, respectively).

Our logistic regression analysis was made on a large population and confirmed that the association of plaque enhancement and symptoms is independent from other major cardiovascular risk factors. Although atherosclerotic plaque neovascularization was identified >70 years ago, its role in plaque vulnerability has only recently gained attention. The VV represent a network of microvasculature that originates primarily in the adventitial layer of large arteries and supplies oxygen and nutrients to the outer layer of the arterial wall. The expansion of the VV is associated with neovascularization related to progression of atherosclerosis. A breach in the medial wall may facilitate the rapid proliferation of microvessels from the adventitia, and exposure to an atherosclerotic environment stimulates abnormal vascular development characterized by disorganized branching with leaky vessels. The change in vessel permeability has been evaluated with MRI by Sun et al. and they found a direct relationship of leaky vessels and increased intraplaque hemorrhage.

Table 1. Baseline Characteristics of the Study Population

<table>
<thead>
<tr>
<th>Characteristic of the plaque, n (%)</th>
<th>No. of participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enhanced</td>
<td>103 (24.1)</td>
</tr>
<tr>
<td>Calcified</td>
<td>202 (47.2)</td>
</tr>
<tr>
<td>Nonenhancing-noncalcified</td>
<td>123 (28.7)</td>
</tr>
</tbody>
</table>

Afib indicates atrial fibrillation; CAD, coronary artery disease; DM, diabetes mellitus; HC, hypercholesterolemia; HTN, hypertension; and Tob, tobacco consumption.

respectively; Fisher exact test). Cardiovascular risk factors were prevalent in this population: hypertension was present in 341 (79.7%), hypercholesterolemia in 134 (31.3%), atrial fibrillation in 63 (14.7%), coronary artery disease in 162 (37.8%), diabetes mellitus in 104 (24.2%), and tobacco consumption in 135 (31.5%; Table 1). On CTA-SI, 103 (24.1%) plaques were described as enhancing, 202 (47.2%) as calcified, and 123 (28.7%) as nonenhancing-noncalcified (Table 1). Symptomatic lesions were detected in 32% (n=33) of the enhancing lesions, 20.8% (n=42) of the calcified plaques, and 17.9% (n=22) of the nonenhancing-noncalcified plaques (Table 1). A χ² test confirmed that the proportion of symptomatic individuals was significantly different among the 3 groups (P=0.03). Given a significant difference between groups, we performed a series of simple-effect pairwise Fisher exact tests, which revealed significant differences between the enhancing and the calcified groups (P=0.03; OR=1.80; 95% CI, 1.05–3.07) and between the enhancing and the nonenhancing-noncalcified groups (P=0.02; OR=2.16; 95% CI, 1.16–4.02). However, there was no significant difference between the calcified and the nonenhancing-noncalcified groups (P=0.57; OR=1.20; 95% CI, 0.68–2.14). The OR data have further strengthened the association between the enhancing group and the presence of acute neurological symptoms.

The multivariate logistic regression analyses showed that the association between VV enhancement of the plaque and symptomatic individuals was significant after controlling for cardiovascular risk factors (P=0.011). Furthermore, this analysis showed that the correlation with symptomatic patients was also statistically significant for hypertension (P=0.021), atrial fibrillation (P=0.027), and coronary artery disease (P=0.019; Table 2).
Because of contrast timing and anatomic location in the vessel wall, the observed regions of high density were attributed to the presence of contrast material in the VV. In this analysis, we distinguished between different types of carotid wall characteristics (enhancing, calcified, nonenhancing-noncalcified) that were readily identifiable in all studies. Interobserver agreement for the rating of the lesions was excellent (κ=0.80). Near-perfect agreement was noted between the visual assessment and the assessment performed with HU measurements, supporting previous trials14 that visual inspection is a sufficient, accurate, and reproducible method. Vulnerable plaque has been an active research topic lately, and other characteristics such as intraplaque hemorrhage and thin fibrous plaque have shown high correlation with neurological symptoms.28,29

Our observation is consistent with previous histological and imaging studies reporting neovascularization in the ICA wall of symptomatic individuals. In a postmortem analysis, Fleiner et al15 found that patients with symptomatic atherosclerosis had greater density of adventitial VV than patients with asymptomatic disease. In addition, their quantitative analysis of angiogenic and inflammatory events in the arterial walls of vulnerable patients showed that hyperplasia of VV was an early sign and macrophage infiltration was a late sign of the development of symptomatic atherosclerosis. Our results favor the early presence of VV enhancement. An early report of imaging of the VV was made in 1999 by Martin et al,15 who detected carotid wall enhancement in atherosclerotic plaques in 4 patients using angiographic sequences as an expression of intraplaque neovascularization. Recently, contrast-enhanced ultrasound, dynamic contrast-enhanced MRI, and CTA have detected enhancement of the VV. Contrast-enhanced ultrasound provides images of the adventitial VV and intraplaque neovascularization in carotid lesions using contrast agent microspheres such as intravascular tracers.14,31 Limitations of this technique include the short half-life of these bubbles in vivo, low resolution,14,31 and poor reproducibility. Dynamic contrast-enhanced MRI offers a potential alternative to ultrasound for characterizing the VV. Kerwin et al13 proposed the contrast transfer constant as a quantitative measurement that corresponds to the extension of the VV. This measurement of VV correlated directly with neovascularature and macrophages on histological specimens. Furthermore, it was associated with C-reactive protein levels (P=0.01) and high-density lipoprotein (P<0.01) and was elevated in active smokers (P=0.02). In a recent, relatively small qualitative study of plaque MR enhancement patterns, Qiao et al36 found that adventitial enhancement was independently associated with increasing frequencies of previous ischemic events (P=0.02). These studies highlight the capability of MRI to detect VV enhancement; however, larger-scale studies may be required to validate their results. To our knowledge, we present the largest series of patients confirming the association of plaque VV enhancement and symptoms in moderate ICA stenosis using CTA.

Although dynamic contrast-enhanced MRI demonstrates high spatial resolution of VV enhancement, this technique is also associated with several technical limitations. The need for neck coils and the relatively long scan times make this examination difficult to perform on acute patients who are on ventilator support, unstable, or agitated, the latter of which may also result in a frequent motion artifact.

CTA is currently the only noninvasive imaging modality that combines high resolution with examination speed for visualizing carotid plaques in the acute setting. Our study confirms the usefulness of CTA in displaying VV enhancement, which correlates with an increased risk for neuroischemic events. In addition, CTA could eventually be used in identifying patients suitable for revascularization as well as a target for medical therapies to stabilize the vulnerable plaque and reduce subsequent cerebrovascular risk.13,34 These results are in line with Romero et al35 who analyzed patients with >70% ICA stenosis on CTA. They demonstrated that patients with carotid wall enhancement were statistically more likely to be symptomatic than those with calcified plaque or no enhancement (OR=3.63; P=0.01). This analysis was performed in an already high-risk group of patients with severe stenosis of >70%.

We are limited in determining the chronicity of the findings that was identified on the CTA-SI and whether some plaques may change in appearance with time. Also, we cannot precisely estimate the risk of developing a future stroke in the different patient subsets. The latter objective may be assessed with a prospective study that would evaluate the predictive value of carotid VV enhancement. Nonactive tobacco consumption included former smokers and never smokers; thus, we cannot establish a difference between former smokers and nonsmokers. Finally, there may be a confounding factor in the interpretation of CTA-SI related to plaques with mixed features; this is more relevant in calcified plaques because dense calcifications can obscure more subtle wall enhancement; however, this factor may be solved in the future with spectral CT imaging in which minimal amounts of enhancement could be detected once there is calcium density removal. Unfortunately, we were unable to evaluate the specimens of the symptomatic operated patients because of the fact that few patients were operated with 50% to 70% stenosis at our institution and the available specimens did not include the carotid artery wall.

Conclusions
Enhancement of the VV significantly correlates with acute neurological symptoms in patients with moderate 50% to 70% ICA stenosis (OR=1.92; 95% CI, 1.17–3.16; P=0.015), VV enhancement is independently associated with recent stroke and may provide additional stratification of stroke risk in patients with moderate stenosis.

Acknowledgments
We thank Adrian Ciobanu, BSc, for database updating.

Disclosures
None.

References


Vasa Vasorum Enhancement on Computerized Tomographic Angiography Correlates With Symptomatic Patients With 50% to 70% Carotid Artery Stenosis
Javier M. Romero, Raffaella Pizzolato, Wendy Atkinson, Anna Meader, Camilo Jaimes, Glenn Lamuraglia, Michael R. Jaff, Ferdinando Buonanno, Josser Delgado Almandoz and Ramon G. Gonzalez

*Stroke*. 2013;44:3344-3349; originally published online October 30, 2013; doi: 10.1161/STROKEAHA.113.002400

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2013 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/44/12/3344

**Permissions:** Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Stroke* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

**Reprints:** Information about reprints can be found online at:
http://www.lww.com/reprints

**Subscriptions:** Information about subscribing to *Stroke* is online at:
http://stroke.ahajournals.org/subscriptions/