Carotid Plaque Pathology
Thrombosis, Ulceration, and Stroke Pathogenesis

Mark Fisher, MD; Annlia Paganini-Hill, PhD; Aldana Martin, HT, HTL; Michele Cosgrove, MD; James F. Toole, MD; Henry J.M. Barnett, MD; John Norris, MD

Background and Purpose—To determine the relationship between ulceration, thrombus, and calcification of carotid artery atherosclerotic plaques and symptoms of ipsilateral or contralateral stroke.

Methods—We compared microscopic plaque morphology from patients with and without stroke symptoms ipsilateral or contralateral to the plaque. Plaques were characterized for ulceration, thrombus, and calcification. We analyzed plaques from 241 subjects: 170 patients enrolled in the Asymptomatic Carotid Atherosclerosis Study (ACAS) and 71 patients enrolled in the North American Symptomatic Carotid Endarterectomy Trial (NASCET); 128 subjects had no history of stroke symptoms, 80 subjects had ipsilateral symptoms, and 33 had contralateral symptoms.

Results—Plaque ulceration was more common in plaques taken from symptomatic patients than those without symptoms (36% versus 14%; \( P<0.001 \)); frequency of ulceration was similar for plaques associated with ipsilateral (34%) and contralateral (42%) symptoms. Thrombus was most common in plaques taken from patients with both ipsilateral symptoms and ulceration. The extent of calcification was unassociated with stroke symptoms.

Conclusion—Carotid plaque ulceration and thrombosis are more prevalent in symptomatic patients. Ulceration is more common in symptomatic patients regardless of side of carotid symptoms, whereas thrombus is associated with ipsilateral symptoms and plaque ulceration. Preoperative identification of carotid ulceration and thrombus should lead to greater efficacy of stroke prevention by carotid endarterectomy. (Stroke. 2005;36:253-257.)

Key Words: atherosclerosis ▪ carotid arteries ▪ stroke

Carotid artery disease is a well-established risk factor for ischemic stroke. Surgical removal of carotid plaques reduces risk of stroke in symptomatic and asymptomatic individuals.1,2 Nevertheless, the relationship between carotid plaque morphology and stroke pathogenesis is poorly understood.

Two large prospective randomized trials have provided important information regarding the role of carotid endarterectomy.1,2 The North American Symptomatic Carotid Endarterectomy Trial (NASCET) and the Asymptomatic Carotid Atherosclerosis Study (ACAS) have shown clinical effectiveness of endarterectomy in substantial groups of patients with carotid artery disease. NASCET has shown benefit for carotid artery surgery in patients with symptomatic disease of 50% or more stenosis; ACAS has shown benefit for carotid artery surgery in patients with symptomatic disease of 60% or more stenosis in a selected population of patients if the surgical morbidity/mortality is <3% and the patient has at least a 5-year prognosis for a healthy life thereafter.

For the coronary vasculature, extensive work has described progression from the early raised lesion to the later complicated plaque.3 Calcification, ulceration, and thrombosis are the major pathologic events in the transformation of the early asymptomatic lesion to the mature plaque producing myocardial ischemia.3 The role of these pathologic events in the development of brain ischemia is uncertain.

Carotid plaques removed from patients undergoing carotid endarterectomy represent a potentially important source of information explaining the roles of critical pathologic events in stroke pathogenesis. Park showed that grossly visible carotid plaque ulceration is more prevalent in patients who have a history of stroke or transient ischemic attack (TIA).4 Rothwell demonstrated that radiographic evidence of carotid plaque surface irregularity in symptomatic arteries was associated with surface irregularity in contralateral arteries.5 We hypothesized that important differences exist between symptomatic and asymptomatic plaques. In this study, we compare the prevalence of ulceration, thrombosis, and calcification in carotid plaques taken from symptomatic and asymptomatic individuals.

Materials and Methods
Carotid artery atherosclerotic plaques were surgically removed from patients enrolled in ACAS and NASCET. Participating NASCET and

See Editorial Comment, pg 257
asymptomatic lesion to the mature plaque producing myocardial ischemia.3 The role of these pathologic events in the development of brain ischemia is uncertain.

Carotid plaques removed from patients undergoing carotid endarterectomy represent a potentially important source of information explaining the roles of critical pathologic events in stroke pathogenesis. Park showed that grossly visible carotid plaque ulceration is more prevalent in patients who have a history of stroke or transient ischemic attack (TIA).4 Rothwell demonstrated that radiographic evidence of carotid plaque surface irregularity in symptomatic arteries was associated with surface irregularity in contralateral arteries.5 We hypothesized that important differences exist between symptomatic and asymptomatic plaques. In this study, we compare the prevalence of ulceration, thrombosis, and calcification in carotid plaques taken from symptomatic and asymptomatic individuals.

Materials and Methods
Carotid artery atherosclerotic plaques were surgically removed from patients enrolled in ACAS and NASCET. Participating NASCET and
ACAS centers sent formalin-fixed carotid artery plaque specimens from patients to the central pathology laboratory at University of Southern California. These plaques were then x-rayed, photographed, and processed; plaques excessively fragmented (>2 pieces) were not subject to further analysis. Microscopic sections were then taken at 3-mm intervals over the 2-cm region of maximum plaque pathology.

Using an eyepiece micrometer, one observer characterized microscopic sections for presence of ulceration and thrombosis. Microscopic sections were analyzed with the observer blinded to the clinical status of the patients whose plaques were being examined. Ulceration was considered present when a depression below, or disruption of, the plaque surface of 560 mm or more resulted in exposure of substantial amount of lipid (either cholesterol crystals or lipid-laden macrophages) to lumen surface (Figure 1). Thrombus was considered present when the lumen surface was covered by thrombotic material larger than 340×110 μm. In preliminary studies, use of these dimensions (by eyepiece micrometer) was found to identify a substantial proportion of atherosclerotic plaques that had presence of ulceration or thrombus. Kappa values calculated for these variables (ulceration and thrombus) were 0.77 and 0.93.

We x-rayed the formalin-fixed plaques to determine the extent of plaque calcification. These images were analyzed by computer using a Quantimet 970 Image Analysis System and the Quips software package (Cambridge Instruments Ltd, United Kingdom). X-ray negatives of plaques were digitized using a video camera and stored as a 512×512 pixel matrix. The image was displayed as a combination of a gray image and a binary overlay representing the detected region. An amend routine was used to measure total plaque area and area of calcification after calibrating against images of known dimension.

Subjects were classified by history of stroke or TIA. We characterized patients as: asymptomatic (ie, lacking ischemic symptoms); ipsilateral symptomatic (ie, symptomatic with history of symptoms ipsilateral to side of endarterectomy); and contralateral symptomatic (ie, symptomatic with history of symptoms contralateral to side of endarterectomy). Percent stenosis was determined by preoperative arteriogram using NASCET criteria. Differences between groups were tested using χ² tests (for categorical variables) and t tests (for continuous variables). Two-sided P<0.05 was considered significant.

### Results

We analyzed carotid artery plaques from 241 subjects, 170 from ACAS and 71 from NASCET. The mean age of subjects was 67 years (range, 48 to 80 years) and 65% were male. There were 128 asymptomatic subjects, 80 ipsilateral symptomatic patients, and 33 contralateral symptomatic patients. For the 113 subjects with symptoms, the median interval between event and endarterectomy was 48 days. Percent stenoses data were available for 230 subjects: median stenoses for asymptomatic, ipsilateral symptomatic, and contralateral symptomatic patients were 75%, 64%, and 67%.

Ulceration was more prevalent in plaques taken from patients with symptoms (ipsilateral or contralateral) than those from asymptomatic patients (Table; 36% versus 14%; P<0.001). Frequency of ulceration was similar for plaques taken from patients with ipsilateral or with contralateral symptoms (34% versus 42%; Figure 2).

Thrombus was only slightly more prevalent in plaques taken from ipsilateral or contralateral symptomatic patients (23%) compared with asymptomatic subjects (18%), and thrombus prevalence did not differ between patients with ipsilateral versus contralateral symptoms (24% versus 21%). However, among patients with ipsilateral symptoms, presence of thrombus and presence of ulcer were strongly associated (P<0.005; Figure 3).

![Figure 1. Photomicrograph, carotid artery cross-section (hematoxylin & eosin, original magnification ×400) a, Carotid artery plaque surface, without ulceration or thrombus. b, Carotid plaque ulceration. c, Carotid plaque thrombus.](image-url)
Stroke Symptoms Versus Plaque Features

<table>
<thead>
<tr>
<th>Stroke Symptoms</th>
<th>Present (n=105)</th>
<th>Absent (n=91)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulceration</td>
<td>36% (41/113)*</td>
<td>14% (18/128)</td>
</tr>
<tr>
<td>Thrombus</td>
<td>23% (26/113)</td>
<td>18% (23/128)</td>
</tr>
<tr>
<td>Calcification area</td>
<td>16±1.1%</td>
<td>19±1.4%</td>
</tr>
</tbody>
</table>

*P<0.001; Values for clarification are mean±SE.

There was no significant association between presence of thrombus or ulceration and interval between symptoms and surgery: subjects with symptoms ≤30 days before endarterectomy had prevalence of ulceration and thrombus of 41% (17/41) and 29% (12/41); for those subjects with symptoms >30 days before endarterectomy, the frequency of ulceration and thrombus was 33% (24/72) and 19% (14/72). Presence of thrombus or ulceration was also unrelated to percent stenosis: presence of ulceration or thrombus for patients with <60% stenosis, 60% to 69% stenosis, 70% to 79% stenosis, or ≥80% stenosis was 39% (19/49), 37% (23/63), 35% (19/55), and 36% (27/74). Median percent calcification, determined for 196 plaques, was similar for plaques taken from asymptomatic (16%), ipsilateral symptomatic (15%), and contralateral symptomatic patients (15%).

Among the 71 NASCET subjects, there was no significant association between ulceration and: (1) hemisphere versus retinal event (P=0.48); (2) lacunar versus nonlacunar event (P=0.72); (3) stroke versus TIA (P=0.89); or (4) presence or absence of ipsilateral infarct on computed tomography (P=0.44). Among the NASCET patients, there was no significant association between thrombus and hemisphere versus retinal event (P=0.84) and presence or absence of ipsilateral infarct on computed tomography (P=0.73). Thrombus was present in 43% (6/14) of patients with lacunar events versus 18% (7/40) of patients with nonlacunar events (P=0.06). Thrombus was also present in 35% (11/32) of patients with stroke versus 18% (7/39) or patients with TIA (P=0.11).

Discussion

We show that carotid plaque ulceration is more prevalent in plaques taken from symptomatic patients. Ulceration prevalence is comparable for both the ipsilateral symptomatic and contralateral symptomatic patient groups. Thrombus, in contrast, is most common in plaques with both ipsilateral symptoms and ulceration. These findings provide important new insights into the pathogenesis of ischemic symptoms from carotid artery atherosclerotic disease.

Plaque ulceration is a complex process that requires chronic increase in shear stress at sites of stenosis and sudden changes in intra-arterial pressure. Both intraplaque inflammation and metalloproteinase activity are initiators of ulceration. Plaque erosions, both superficial and deep, are invariably associated with activated macrophages and T lymphocytes. Plaque macrophages tend to localize to high-shear regions of plaques, in contrast to smooth muscle cells that are more prominent in low-shear regions. Other manifestations of inflammation, including expression of inflammatory cytokines, are more common in symptomatic carotid plaques. Demonstrations of the Gram-negative organism Chlamydia pneumoniae in atherosclerotic lesions, including carotid plaques, suggest a linkage between systemic infection and focal atherosclerotic inflammatory changes.

Metalloproteinases within plaques are also linked to plaque ulceration. Mast cell-derived proteases appear to have an important role in the activation of metalloproteinases, resulting in degradation of plaque wall and subsequent ulceration. Mast cells, along with smooth muscle cells and macrophages, also appear to have a role in plaque calcification. The current study found no relationship between the extent of plaque calcification and symptoms, consistent with previous work.

Atherosclerotic plaques have a predisposition to thrombosis. Previous work has demonstrated a profound procoagulant milieu within plaques. Plasminogen activator inhibitor-1, the primary inhibitor of tissue plasminogen activator, is overexpressed within plaques, creating a net antifibrinolytic effect. Tissue factor, the primary generator of the coagulation cascade, is expressed by critical elements of atherosclerotic lesions. The extent of tissue factor activity in plaques is partially dependent on the extent and distribution of tissue factor pathway inhibition within plaques. Tissue factor within plaques is localized to areas of extensive lipid deposition, particularly lipid-laden macrophages (foam cells) and cholesterol crystals. Note that our definition of microscopic ulceration requires presence of significant amount of lipid on the lumen surface. Therefore, plaque ulceration, as observed in this study, represents an important site for thrombus formation on the plaque surface.

Figure 2. Plaque ulceration versus stroke symptoms. Plaque ulceration was significantly (P<0.001) more frequent in patients with ipsilateral symptoms (34%, 27/80), contralateral symptoms (42%, 14/33), or both ipsilateral and contralateral symptoms (36%, 41/113) compared to patients without symptoms (14%, 18/128). (Y-axis is percent, *P<0.001).
Based on findings presented herein, as well as previous observations, we suggest that patients destined to be symptomatic from carotid artery disease are more likely to first have plaque ulceration, which tends to occur irrespective of what ultimately will be the side of brain ischemia. Ulceration thus represents an important substrate from which thrombus and symptoms may later arise. Thrombus, superimposed on ulceration, appears to be important for production of brain ischemia. The ischemic episode itself may be produced by microembolism or by hemodynamic insufficiency.

We show that carotid plaque ulceration and thrombus are associated with different subsets of “symptomatic” patients. Ulceration is more common in patients with symptoms either ipsilateral or contralateral to the plaque; thrombus is more common in patients with both ipsilateral symptoms and plaque ulceration. These 2 patient subsets are important in helping to define distinctions between “symptomatic patients” and “symptomatic plaques.” This distinction has typically depended on presence (or absence) of symptoms ipsilateral to the lesion. A carotid artery atherosclerotic plaque, in a patient with only contralateral symptoms of brain ischemia, may sometimes be considered “asymptomatic”; a patient with such a lesion may be grouped with patients who are completely asymptomatic. Our data demonstrate important pathological differences between plaques from patients with and without symptoms, regardless of symptom laterality. Therefore, for future trials of therapy of carotid artery disease, it may be appropriate to consider carotid plaques with contralateral symptoms as “symptomatic.”

Our study is limited by a number of potentially important confounding factors. We evaluated surgical specimens that all had longitudinal incisions, thus creating the potential for artifact. This latter possibility was limited, but not eliminated, by the blinded analysis of nonfragmented plaques. Also note that only a limited number of ACAS and NASCET centers participated in this study, and plaques were typically not sent in consecutive series; for example, excessively fragmented specimens were usually not submitted for analysis. Moreover, these plaques were often not removed from patients chronologically near the time of symptoms. This interval between symptoms and surgery may have contributed to the presence of plaque ulceration or thrombus in a minority of our plaques. Note, however, that our criteria for presence of ulceration or thrombus required a substantial-sized lesion; a morphometric requirement of smaller lesion dimensions may have resulted in a higher proportion of plaques deemed abnormal. Finally, our analysis did not include intraplaque hemorrhage; the latter has recently been described as an important contributor to atherogenesis and plaque destabilization.

In conclusion, carotid plaque ulceration and thrombosis are more prevalent in symptomatic patients. Ulceration is more common in symptomatic patients regardless of side of carotid symptoms, whereas thrombus is associated with ipsilateral symptoms and plaque ulceration. Preoperative identification of carotid ulceration and thrombus by either ultrasound or arteriography is currently quite limited, and improvements in the relevant technologies should lead to greater efficacy of stroke prevention by carotid endarterectomy.

Appendix

Participating ACAS Centers
Albuquerque VA Medical Center, New Mexico; University of Arizona Health Sciences Center; Bowman Gray School of Medicine, Winston-Salem, North Carolina; Columbia Presbyterian Medical Center, New York; East Orange Medical Center, New Jersey; Francis Scott Key Medical Center, Baltimore, Maryland; Harbin Clinic, Rome, Georgia; Henry Ford Hospital, Detroit, Michigan; Hershey Medical Center, Pennsylvania; University of Kentucky, Lexington, Kentucky; Hospital de l'Enfant-Jesus, Quebec; Little Rock VA Medical Center, Arkansas; Loyola University Medical Center, Maywood, Illinois; Marshfield Medical Clinic, Marshfield, Wisconsin; New England Medical Center, Boston, Massachusetts;

Figure 3. Plaque thrombus versus stroke symptoms and ulceration. Plaque thrombus was significantly (P<0.005) more frequent in patients with ipsilateral symptoms and plaque ulceration (44%, 12/27) compared to patients with ipsilateral symptoms and no plaque ulceration (13%, 7/53). Plaque thrombus and ulceration coexisted in 28% (5/18) of asymptomatic patients and in 21% (3/14) of patients with contralateral symptoms. Plaque thrombus without ulceration was found in 16% (18/110) of asymptomatic patients and in 21% (4/19) of patients with contralateral symptoms. (Y-axis is percent, *P<0.005).
Ochsner Clinic, New Orleans, Louisiana; Oregon Health Sciences University School of Medicine, Portland, Oregon; Richmond VA Medical Center, Richmond, Virginia; Saint John’s Mercy Medical Center, St. Louis, Missouri; San Diego VA Medical Center, San Diego, California; Singing River Hospital, Pascagoula, Mississippi; Sunnybrook Health Science Center, Toronto, Ontario; UCLA Medical Center; Virginia Mason Research Center, Seattle, Washington. A full list of collaborators from these centers is provided in reference 2.

Participating NASCET Centers
University of Illinois Hospital Chicago, Illinois (C. Helgason); Northwestern University Chicago, Illinois (B. Cohen); Ohio State University Columbus, Ohio (A. Slikvra) Dartmouth-Hitchcock Medical Center Lebanon, New Hampshire (A. Reeves); University of Texas Health Sciences (J. Grotta); UCLA School of Medicine, California (W. Moore); USC School of Medicine Los Angeles, California (M. Fisher); Madison VAMC Madison, Wisconsin (C. Acher); Marshfield Medical Clinic Marshfield, Wisconsin (P. Karanjia); Mississauga Hospital, Mississauga, Ontario (G. Sawa); Hospital de L’Enfant Jesus Quebec City, PQ (D. Simard); Richmond Eye & Ear Hospital Richmond, Virginia (J. Harbison); VA Medical Center Syracuse, New York (A. Culebras); Sunnybrook Health Science Center Toronto, Ontario (J. Norris); Vancouver General Hospital UBC Health Sciences Center Vancouver, BC (P. Teal).

Acknowledgments
This work was supported by National Institutes Health NS20989, NS06655, and NS24456. We thank Paul S. Reehal, MD for his assistance in preparing this manuscript.

References
9. Van der Wal AC, Becker AE, van der Loos CM, Das PK. Site of intimal rupture or erosion of thrombosed coronary atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology. Circulation. 1994;89:36–44.

Editorial Comment
Plaque Pathology and Patient Selection for Carotid Endarterectomy

Experienced carotid surgeons know that when performing a carotid endarterectomy, it is important to avoid excessive manipulation of the carotid bifurcation during dissection, especially when the patient has had recent symptoms from an “active” plaque. This study by Fisher et al comparing the pathology of carotid plaques obtained from the asymptomatic carotid endarterectomy trial, ACAS, to those taken from the symptomatic trial, NASCET, helps explain the danger. Plaques resected from symptomatic patients are more frequently ulcerated and have attached thrombus compared with asymptomatic plaques and, therefore, are at greater risk to embolize clot and atherosclerotic debris into the cerebral circulation during surgical mobilization. During carotid endarterectomy, the tube-like carotid plaque is cut length-wise and not infrequently torn, preventing a highly accurate gross or microscopic assessment. This might explain why the associations between ulcers, thrombus, and ipsilateral symptoms were not found to be even stronger. In the study reported...
here, ulceration has a similar frequency in either carotid of a patient with unilateral symptoms, and thrombus was relatively common in plaques not causing symptoms, being present in 21% of plaques in which the symptoms were from the contralateral carotid and 18% of asymptomatic patients. Trends detected in this study (not reaching statistical significance) included the greater prevalence of plaque thrombus when symptoms were closer to the time of surgery (29% for symptoms ≤30 days versus 19% for symptoms >30 days from surgery) and the greater prevalence of thrombus in those symptomatic patients with stroke (35%) than those with transient ischemic attack (18%).

Is there anything in this study we can apply to patients in the clinic? As suggested, it might partly explain the frequent observation that carotid endarterectomy seems safer in asymptomatic than symptomatic patients.1 Does it in any way help us to better-select patients for carotid endarterectomy? The authors suggest that asymptomatic plaques found opposite to symptomatic plaques in the same patients are more commonly ulcerated with attached thrombus and, therefore, perhaps more dangerous. But they remind us also that ulceration and intraluminal thrombus are sometimes difficult to detect with carotid imaging, and this is particularly true with carotid ultrasound and contrast magnetic resonance angiography. So, I am not sure how further ahead we are with the information provided in this report.

Is it in fact possible to determine which asymptomatic carotid plaques have the greatest risk of causing future stroke, therefore justifying consideration of prophylactic repair? Suggested risk factors include male sex,2 ipsilateral brain infarction on brain imaging,3 plaque ulceration detectable on angiography,4–8 the presence of an occluded contralateral carotid artery,9 a stenosis that worsens over time,10 a partly echoluent or heterogenous (“soft”) plaque or evidence of intraplaque hemorrhage on ultrasound,10–14 carotid wall “stiffness” or distensibility during the cardiac cycle,15 and the presence of microemboli detected distal to the plaque on transcranial Doppler.16 Of course, younger patients stand to benefit more from carotid endarterectomy than an elderly person with asymptomatic stenosis. Interestingly, higher degrees of arterial narrowing caused by plaque, an important risk factor for symptomatic stenosis,17 and an intuitive and commonly considered risk factor for asymptomatic patients, was not found to correlate with surgical benefit in either ACAS18 or the recently published Asymptomatic Carotid Surgery Trial (ACST).19 Perhaps in the future, chemical composition of carotid plaques as determined by magnetic resonance spectroscopy or molecular markers in either the plaque or serum will be found to correlate with stroke risk,20 but a powerful relationship would be required for a significant correlation to exist, given the overall relatively benign natural history of asymptomatic plaques.

We continue to consider asymptomatic carotid stenosis to be an “uncertain” indication for carotid endarterectomy under any circumstance, requiring individual patient assessment and selection, and surgery should be offered only by expert surgeons with low complication rates.21
In the article by Fisher et al1, “Carotid plaque pathology: thrombosis, ulceration and stroke pathogenesis,” which appeared in the February 2005 issue of Stroke, there was a typographical error on page 254. In the paragraph beginning, “Using an eyepiece micrometer...” the plaque surface of 560 mm should have been 560 μm. The authors apologize for this error.


Stroke is available at http://www.strokeaha.org DOI: 10.1161/01.str.0000186207.43801.28