Juxtalumenal location of plaque necrosis and neoformation in symptomatic carotid stenosis

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Purpose: The structural features that underlie carotid plaque disruption and symptoms are largely unknown. We have previously shown that the chemical composition and structural complexity of critical carotid stenoses are related to plaque size regardless of symptoms. To further determine whether the spatial distribution of individual plaque components in relation to the lumen corresponds to symptomatic outcome, we evaluated 99 carotid endarterectomy plaques.

Methods: Indications for operation were symptomatic disease in 59 instances (including hemispheric transient ischemic attack in 29, stroke in 19, and amaurosis fugax in 11) and angiographic asymptomatic stenosis >75% in 40. Plaques removed after remote symptoms beyond 6 months were excluded. Histologic sections from the most stenotic region of the plaque were examined using computer-assisted morphometric analysis. The percent area of plaque cross-section occupied by necrotic lipid core with or without associated plaque hematoma, by calcification, as well as the distance from the lumen or fibrous cap of each of these features, were determined. The presence of foam cells, macrophages, and inflammatory cell collections within, on, or just beneath the fibrous cap was taken as an additional indication of plaque neoformation.

Results: The mean percent angiographic stenosis was 82% ± 11% and 79% ± 13% for the asymptomatic and symptomatic groups, respectively ($p > 0.05$). The necrotic core was twice as close to the lumen in symptomatic plaques when compared with asymptomatic plaques (0.27 ± 0.3 mm vs 0.5 ± 0.5 mm; $p < 0.01$). The percent area of necrotic core or calcification was similar for both groups (22% vs 26% and 7% vs 6%, respectively). There was no significant relationship to symptom production of either the distance of calcification from the lumen or of the percent area occupied by the lipid necrotic core or calcification. The number of macrophages infiltrating the region of the fibrous cap was three times greater in the symptomatic plaques compared with the asymptomatic plaques (1114 ± 1104 vs 385 ± 622, respectively, $p < 0.009$). Regions of fibrous cap disruption or ulceration were more commonly observed in the symptomatic plaques than in the asymptomatic plaques (32% vs 20%). None of the demographic or clinical atherosclerosis risk factors distinguished between symptomatic and asymptomatic plaques.

Conclusions: These findings indicate that proximity of plaque necrotic core to the lumen and cellular indicators of plaque neoformation or inflammatory reaction about the fibrous cap are associated with clinical ischemic events. The morphologic complexity of carotid stenoses does not appear to determine symptomatic outcome but rather the topography of individual plaque components in relation to the fibrous cap and the lumen. Imaging techniques that precisely resolve the position of the necrotic core and evidence of inflammatory reactions within carotid plaques should help identify high-risk stenoses before disruption and symptomatic carotid disease. (J Vasc Surg 1997;26:585-94.)

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Table I. Clinical data (n = 99)

	All patients	*Symptomatic*	*Asymptomatic*	*p*
Patient number		59	40	NS
Male:female	58:41	36:23	22:18	NS
Age (yr)	71 ± 8	70 ± 8	71 ± 9	NS
Symptoms		TIA: 40 cases Stroke: 19 cases		
Intervals (wk)*		7 ± 11		
Diabetes mellitus	28	17	11	NS
Hypertension	78	46	32	NS
Hypercholesterolemia	10	6	4	NS
Smoking	64	42	22	NS
Coronary artery disease	38	25	13	NS

*Interval from symptom to operation.

Atherosclerotic plaque disruption is recognized as a salient feature critical to the development of clinical ischemic manifestations in both the coronary and carotid circulations.[1-5] Although the structural features of advanced atherosclerotic plaques as well as the biomechanical forces imposed on them have been extensively studied, the relevance of particular plaque morphologic features to fibrous cap erosion and plaque complications such as thrombosis, embolization, and intraplaque hematoma has yet to be elucidated. We have previously demonstrated that symptomatic and asymptomatic critical carotid stenosis have similar morphologic and chemical features. Morphologic complexity was related to plaque size regardless of symptoms.[6] Conversely, other investigators have observed that carotid artery plaques in symptomatic patients contained more soft cholesterol amorphous debris and hemorrhage and less collagenous material when compared with asymptomatic patients.[7,8] A recognized limitation inherent to the aforementioned studies is the lack of precise quantitation of the individual components of the plaque and inconsistencies with regard to the selected sampling site.

More recently, the role of inflammatory cell infiltrate has been invoked in unstable, symptom-producing carotid and coronary plaques[9-11] and related to degradation of the fibrous cap and plaque neoformation. A variety of biomechanical factors have also been postulated to play a role in plaque disruption. These include mechanical stresses associated with hemodynamic wall shear or pressure fluctuations.[12-15] Although advanced symptomatic and asymptomatic carotid plaques contain similar fibrous, necrotic, and calcific components, we postulated that the spatial distribution of these individual components in relation to the lumen and the extent of inflammatory cell infiltration, and in particular macrophage foam cell connoting plaque neoformation in the abluminal fibrous cap, could discriminate between symptomatic and asymptomatic outcome. We therefore examined a large number of symptomatic and asymptomatic carotid plaques with similar degrees of stenosis that had been removed at endarterectomy. In view of the relationship between plaque complexity, that is, the presence of a diversity of plaque components and plaque size or degree of stenosis, we evaluated histologic sections from the most stenotic region of these plaques with respect to the relative quantity and location of the various plaque components and the proximity of these elements to each other and the lumen surface. We further measured the degree of macrophage infiltration in and about the fibrous cap as a measure of likely plaque neoformation. This investigation allowed for a more objective comparative analysis of the notable morphologic features in endarterectomized symptomatic and asymptomatic plaques.

MATERIALS AND METHODS

Carotid endarterectomy specimens. Specimens were obtained from 99 patients (58 men and 41 women) who underwent carotid endarterectomy for treatment of high-grade carotid stenosis. The patients' mean age was 71 years, with a range of 48 to 90 years. Ninety-six patients had undergone unilateral carotid endarterectomy, and three had undergone bilateral carotid endarterectomy. In all patients, aortic arch and four-vessel arteriography was performed before surgery, and lumen stenosis was calculated from diameter measurements at the stenosis and at the nonstenotic distal internal carotid artery. Clinical risk factors for atherosclerosis were recorded for all patients.

Carotid endarterectomy was performed for symptomatic carotid disease in 59 instances. Forty patients had had a transient ischemic attack within 20 weeks before the operation, 19 patients had had a stroke

more than 1 month before the operation. In 40 instances, endarterectomy was performed for high-grade asymptomatic carotid stenosis (Table I). Plaques were endarterectomized using a standard open technique to minimize histopathologic artifacts. The harvested specimen included plaque involving the distal common carotid artery in continuity with the bifurcation and its extension into the internal and external carotid arteries. Morphologic evaluation of the harvested plaques was conducted by an experienced angiopathologist (S.G.) and were carefully scrutinized for disruptions related to operative manipulation. The depth of the arteriotomy was limited to the outer media whenever technically feasible to minimize disruption of the luminal surface at the regions of greatest plaque burden. The arteriotomy was uniformly carried into the lumen of the internal carotid artery to adequately visualize the plaque's terminal endpoint.

The fresh endarterectomy specimen was rinsed gently in normal saline solution to remove surface blood; inspected for macroscopic evidence of hemorrhage, ulceration, and thrombosis; and then photographed. Each specimen was immersed in 10% formalin fixative and subsequently in a decalcifying solution. The specimens were sectioned perpendicular to the axial centerline of the common and internal carotid artery segments at 0.5 cm intervals, and the blocks were numbered in sequence as shown in Fig. 1. For most of the specimens. Seven to eight blocks were available. Each block was processed through paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin and by the Gomori trichrome aldehyde fucshin and Weigert von Gieson procedures for distinguishing connective tissue fibers and cells.

Sections were examined to characterize the following histologic features at each level: necrosis, calcification, fibrous cap erosion or ulceration, hemorrhage, thrombosis, and evidence of macrophage and inflammatory cell reactions about the fibrous cap. Sections at each level contained characteristic features of plaque composition, but these were most abundant and varied at the level of the largest plaque. The maximum plaque area often corresponded to the level of maximum cross-sectional stenosis as determined by morphometric analysis and usually occurred in the proximal region of the sinus. Although the principle findings reported here were similar at all levels, they were most striking at the level with the largest plaque regardless of absolute degree of stenosis. The present report therefore deals with the morphometric findings in the sections with the largest plaque area of each endarterectomy specimen. Special plaque morphologic features and complexity at each level with respect to distance from the bifurcation will be addressed in future studies.

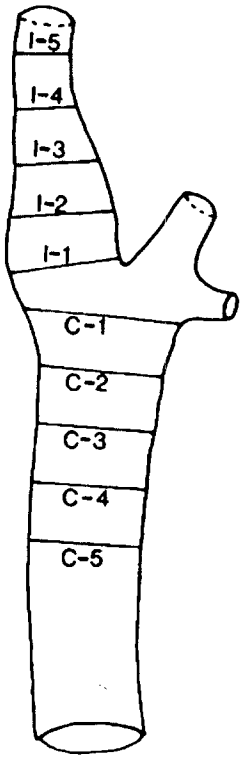

Fig. 1. Diagram of section levels in carotid bifurcation specimens.

Morphometric analysis of plaque components. The selected sections were photographed and used to produce magnified (×22) color prints. Contours of the lumen, internal elastic lamina, fibrous cap, regions of necrosis, and calcification were traced on the prints, and dimensions were computed by using on-line computer-assisted program.

The fibrous cap was defined as the immediate subendothelial region and usually consisted of a compact layer of smooth muscle cells and connective tissue fibers. More extensive fibrous zones without clear demarcation on the selected sections were measured on the basis of estimates made from views of adjacent sections. The necrotic core usually occupied deeper regions of the plaque and was composed of amorphous debris and cholesterol clefts. Distinct calcific deposits appeared as dark blue, sharply demarcated regions in the hematoxylin and eosin stains. Fine punctate calcific deposits often occurred in association with myxomatous regions. These were not quantitated for the present study.

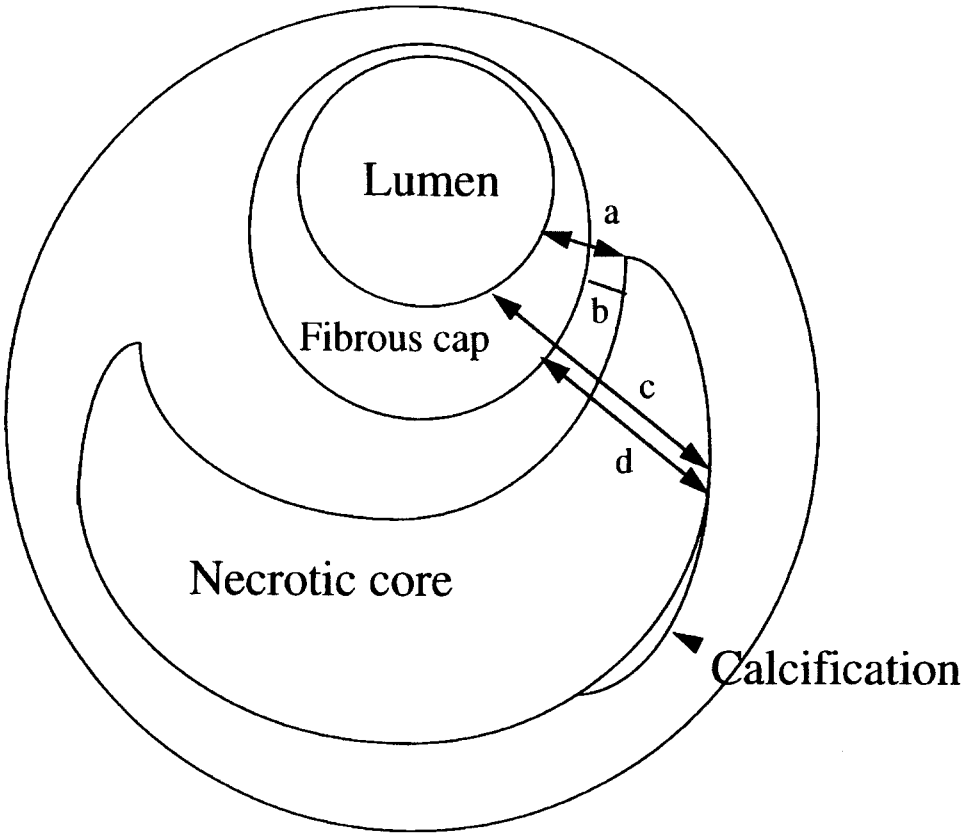

Fig. 2. Illustration represents computer-assisted morphometric analysis of plaque cross-sections. As shown, individual plaque components were contour-traced and distance between each component and fibrous cap and lumen were measured. Distance between necrotic core and fibrous cap or lumen is represented by *a* and *b*. Distance between calcification and fibrous cap or lumen is represented by *c* and *d*.

The absolute area of each component was computed from the contour tracings. In addition, the percent area of each component was calculated using the following formula: percent area = (area of a component/total area of plaque section) × 100. The shortest distance from the necrotic core and calcification regions of the lumen or fibrous cap and the minimum thickness of the fibrous cap overlying the necrotic core were also measured (Fig. 2).

Measurement of presumed plaque neoformation; macrophage infiltration. Because foam cells of macrophage origin are associated with early plaque evolution and necrotic core formation, we determined the number of macrophages in and about the fibrous caps of 45 of the asymptomatic (n = 22) and symptomatic (n = 21) sections. Immunohistochemical staining for macrophages was carried out on paraffin-embedded sections mounted on poly-L-lysin–coated slides and air dried overnight. Sections were deparafinized by immersing the slides in two changes of xylene for 15 minutes each and then rehydrated in 100%, 95%, and 75% ethanol for 3 minutes each. After rehydration, sections were washed in phosphate-buffered saline solution. Endogenous peroxidase activity was blocked with 3.0% hydrogen peroxide (H_2O_2) in phosphate-buffered saline solution. Sections were pretreated with 10% goat serum for 20 minutes and then incubated with mouse antihuman macrophage antibody (1:30 dilution) for 1 hour at 37° C. The avidin-biotin-immunoperoxidase method was used to identify the macrophages according to the supplier's guidelines (Vector, Calif.). Incubation with 0.1% 3',3'-diaminobezidine and H_2O_2 at room temperature for 5 to 10 minutes produced a brown reaction product. Sections were lightly counterstained with hematoxylin to visualize nuclei. The number of macrophages was counted in five successive sections in the region of the plaque with largest area. The total number of macrophages per unit area was determined in the

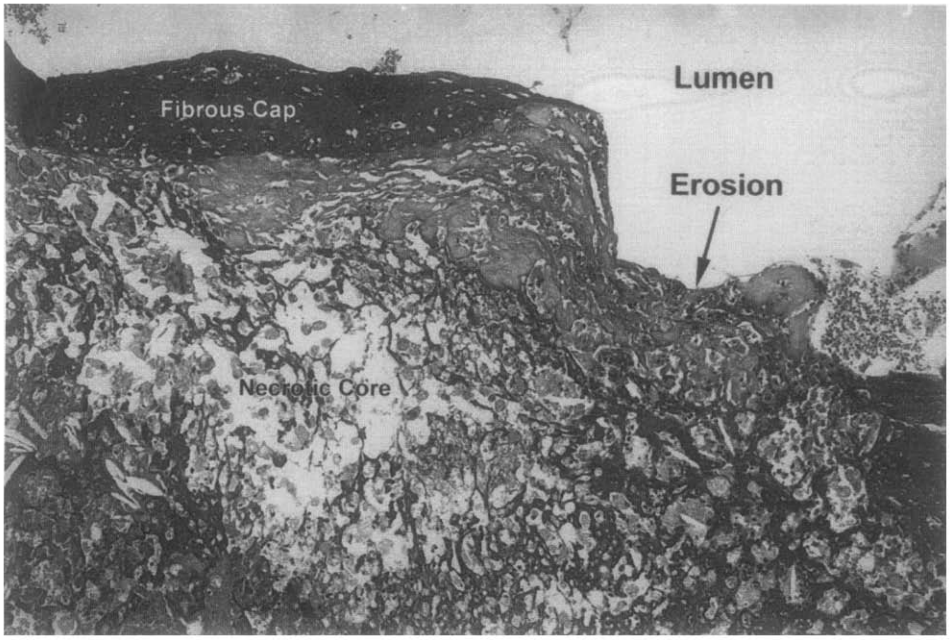

Fig. 3. Photomicrograph of a histologic section (original magnification, ×25) in a symptomatic plaque representing fibrous cap thinning and erosion with exposure of the necrotic core to the lumen.

fibrous cap regions overlying the necrotic core. These were identified as the regions of the plaque between the necrotic core and lumen composed characteristically of smooth muscle cells, extracellular matrix, and connective tissue fibers.

Statistical analysis. Results were expressed as mean ± SD. All morphometric data and cellular counts of macrophage infiltration were compared between asymptomatic and symptomatic plaques using paired Student's t tests. χ^2 analysis was used to compare clinical demographics between both groups. Differences were considered significant at the $p < 0.05$ level.

RESULTS

Clinical features and risk factors. Patients who underwent endarterectomy for symptomatic carotid atherosclerosis or asymptomatic severe carotid stenoses had similar clinical findings (Table I). Although a trend toward greater exposure to atherosclerosis risk factors was noted in symptomatic patients compared with asymptomatic patients, the differences were not statistically significant.

Histopathologic analysis. Sections of large plaques from both symptomatic and asymptomatic patients exhibited microanatomic complexity. These included the presence of necrotic and calcific regions with an overlying fibrous cap or fibrous regions of varied thickness. Histopathologic features associated with plaque disruption, notably ulceration, plaque hematoma, fresh surface thrombosis, and inflammation, were observed. Regions of actual fibrous cap disruption or ulceration were observed in 32% of specimens associated with symptoms (19 of 59) and in only 20% of those without symptoms (eight of 40). The prevalence of recent intraplaque hematoma and surface thrombosis was not different between symptomatic and asymptomatic plaques (19% and 8% vs 18% and 5%, respectively). Fig. 3 is representative of a symptomatic plaque. There is fibrous cap erosion with exposure of the underlying necrotic core to the lumen. Fig. 4 is from a section of an asymptomatic plaque. A thick fibrous cap overlies and isolates the necrotic core from the lumen.

Morphometric analysis. The mean plaque area of all specimens was 35 ± 12 mm² (range, 13 to 65). Mean percent cross-sectional stenosis was 81% ± 12% (range, 42% to 99%). The mean percent necrotic core area was 21% ± 18% (range, 0% to 77%), and the mean percent calcification area was 7.0% ± 11% (range, 0.4% to 40%). The mean percent stenosis was 79% ± 13% (range, 54% to 99%) in symptomatic plaques and 82% ± 11% (range, 42% to 99%) in asymptomatic plaques ($p > 0.05$, NS).

The mean percent area of necrotic core and of calcification were not significantly different in symp-

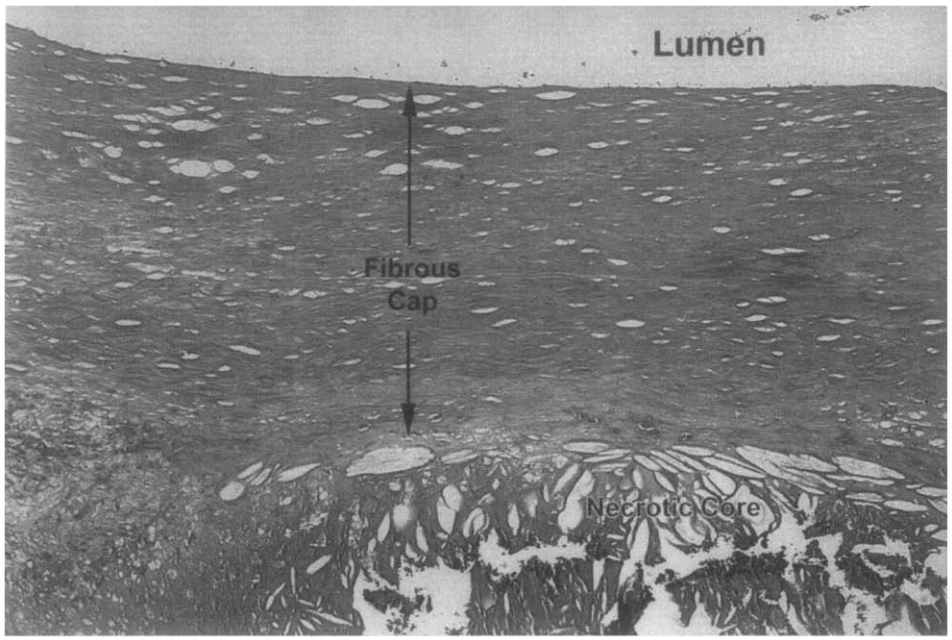

Fig. 4. Photomicrograph (original magnification ×15) illustrates well-developed fibrous cap isolating plaque necrotic core from lumen in asymptomatic plaque.

Table II. Plaque component area

	All patients (n = 99)	*Symptomatic (n = 59)*	*Asymptomatic (n = 40)*	*p*
Plaque area (mm^2)	35% ± 12%	35% ± 11%	35% ± 13%	NS
Percent stenosis	81% ± 12%	79% ± 13%	82% ± 11%	NS
Percent necrotic core	21% ± 18%	22% ± 16%	26% ± 18%	NS
Percent calcification	7% ± 11%	7% ± 10%	6% ± 10%	NS
Percent fibrous cap	10% ± 6%	11% ± 6%	10% ± 6%	NS

Values expressed as mean ± SD of mean.

tomatic and asymptomatic plaques. Values were 22% ± 16% vs 26% ± 18% and 7% ± 10% vs 6% ± 10%, respectively. Similarly, the mean percent fibrous cap area was not different for symptomatic and asymptomatic plaques (11% ± 6% vs 10% ± 6%, respectively; Table II).

Distance between major plaque components and fibrous cap or lumen. For all of the sections, the mean distance between necrotic core and fibrous cap was 0.2 ± 0.3 mm (range, 0 to 1.2 mm; Table III). The distance between the necrotic core and the lumen was 0.4 ± 0.7 mm (range, 0 to 1.4 mm). The mean distance between the calcification and the fibrous cap was 0.5 ± 0.9 mm (range, 0 to 4.4 mm); the mean distance between the calcification and the lumen was 0.8 ± 1.0 mm (range, 0 to 5.4 mm). The mean distance between the necrotic core and the fibrous cap was significantly less in symptomatic plaques when compared with asymptomatic plaques (0.05 ± 0.2 mm vs 0.20 ± 0.3 mm; $p < 0.01$). A scattergram of these values is represented in Fig. 5, *A*. The necrotic core was twice as close to the lumen in symptomatic plaques (0.27 ± 0.3 mm) compared with asymptomatic plaques (0.50 ± 0.5 mm; $p < 0.01$). A scattergram of these values is represented in Fig. 5, *B*. The mean distance between the calcification and the fibrous cap was 0.55 ± 1.1 mm (range, 0 to 4.4 mm) in symptomatic plaques and 0.05 ± 0.3 mm (range, 0 to 3.4 mm) in asymptomatic plaques. The mean distance between the calcification and the lumen was 0.78 ± 1.0 mm (range, 0 to 5.3 mm) and 0.27 ± 1.0 mm (range, 0 to 5.3 mm) in symptomatic and asymptomatic plaques, respectively. Differences for calcification localization were not significant. The minimum thickness of the fibrous cap was measured in the plaque sections that were used for macrophage

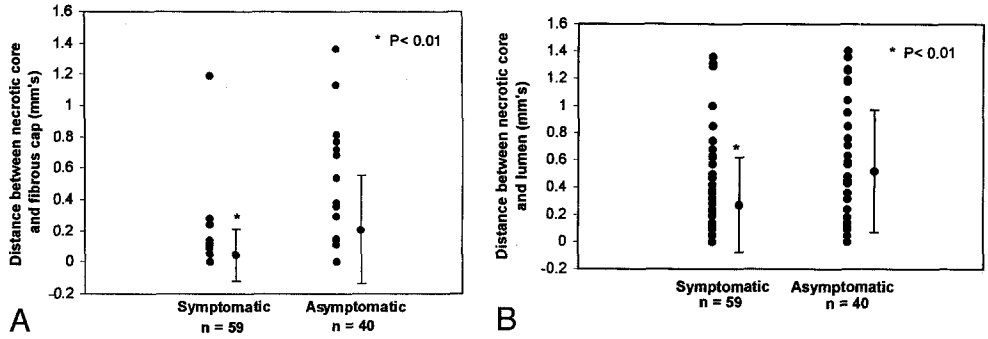

Fig. 5. Scattergram represents values of the distance between the necrotic core and the fibrous cap **(A)** and the lumen **(B)**.

Table III. Distance of plaque components from fibrous cap and lumen

	All patients (n = 99)	*Symptomatic (n = 59)*	*Asymptomatic (n = 40)*	*p*
N1 (mm	0.2 ± 0.3	0.05 ± 0.2	0.20 ± 0.3	0.01
N2 (mm)	0.4 ± 0.7	0.27 ± 0.3	0.50 ± 0.5	0.01
C1 (mm)	0.5 ± 0.9	0.55 ± 1.1	0.05 ± 0.3	NS
C2 (mm)	0.8 ± 1.0	0.78 ± 1.3	0.27 ± 1.0	NS

N1, Distance between necrotic core and fibrous cap; *N2,* distance between necrotic core and lumen; *C1,* distance between calcification and fibrous cap; *C2,* distance between calcification and lumen.
Values expressed as mean ± SD.

characterization. The minimum fibrous cap thickness was significantly less for the symptomatic plaques (0.2 ± 0.2 mm) compared with asymptomatic plaques (0.4 ± 0.4 mm; $p < 0.006$).

Macrophage infiltration. The number of macrophages infiltrating the region of the fibrous material overlying the necrotic core was significantly greater in symptomatic plaques compared with asymptomatic plaques (1144 ± 1104 vs 385 ± 622, respectively; $p < 0.009$). Fig. 6 depicts macrophage infiltration in the fibrous cap region.

DISCUSSION

The mechanisms that underlie plaque instability may involve biologic factors that are intrinsic to plaque structure and biomechanical factors that induce structural breakdown or specific cellular responses. The purpose of this study was to further define and quantitate individual structural components at the level of largest plaque burden of symptomatic and asymptomatic carotid bifurcation endarterectomy specimens and to determine their spatial distribution in relation to the fibrous cap and lumen. The rationale for this analysis was to asses whether predominantly necrotic or calcific plaques were more closely associated with symptoms or whether the position of these components in relation to the fibrous cap and lumen were more important determinants of clinical outcome. We also investigated the association between presumed plaque neoformation, as represented by macrophage infiltration in and about the fibrous cap, and fibrous cap thinning and cerebral ischemic events.

We found that large symptomatic and asymptomatic plaques, often highly stenotic, possess remarkably similar histopathologic features with regard to the presence of necrosis, calcification, fibrous cap ulceration, hemorrhage, and surface thrombosis. Although intraplaque hemorrhage hematoma and surface thrombosis have been regarded by other investigators as cardinal features of symptomatic plaques,[17] such a finding is not substantiated in the current study. This may be related in part to the variable interval between histologic examination of the endarterectomy specimen and neurologic symptoms. These observations substantiate our previous findings[6] regarding the morphologic and chemical similarity between symptomatic and asymptomatic critical stenoses.[11] Several new findings have, however, emerged from this current analysis that help to identify structural features associated with plaque disruption and symptoms.

It is evident from our findings that the proximity of the necrotic core to the overlying fibrous cap and

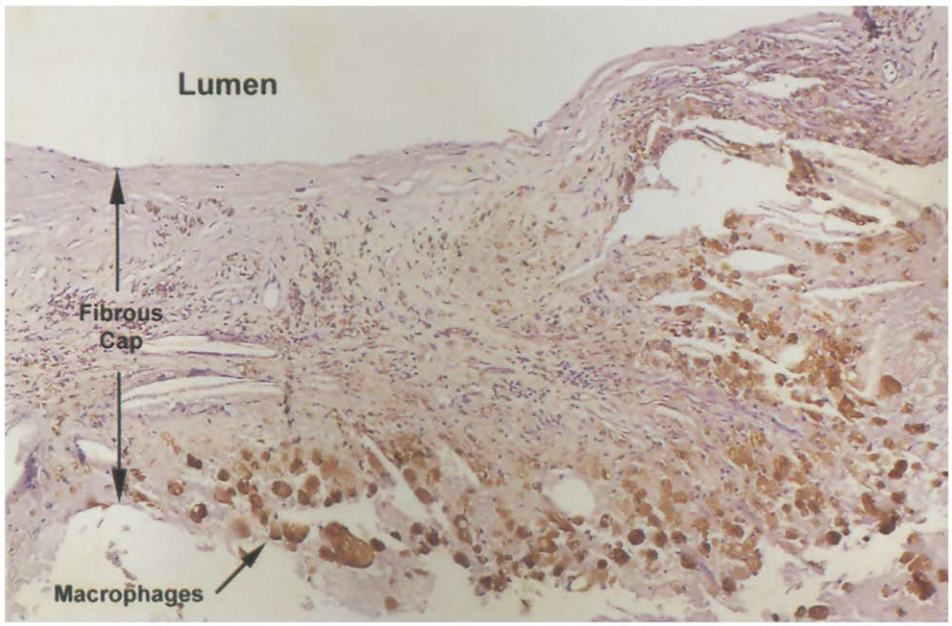

Fig. 6. Immunohistochemical staining of macrophages infiltrating the fibrous cap using mouse antihuman macrophage antibody (original magnification, ×50).

lumen, rather than its absolute or percent cross-sectional area, is a striking feature of symptomatic plaques. In symptomatic plaques, the necrotic core was twice as close to the lumen when compared with asymptomatic plaques, whereas the degree or location of calcification had little effect. Thus the spatial relationship among the matrix and necrotic components appear to be most important in defining likely plaque stability than the content of these elements. These findings likely represent the dynamic equilibrium that exists among factors that favor fibrogenesis versus those that induce matrix degradation.[16-21]

Symptomatic plaques also exhibited a greater degree of macrophage infiltration in and about the fibrous cap and were associated with fibrous cap thinning and erosion, implicating an ongoing induction of plaque formation or an inflammatory or immune-mediated response as a factor in plaque instability. Other investigators have also noted that ruptured plaques are infiltrated by foam cells, especially in regions where the fibrous cap is thinnest.[2,3,10,11,22]

Foam cells derived from mononuclear phagocytes in atherosclerotic plaques are known to elaborate a number of matrix metalloproteinases, such as interstitial collagenase, gelatinase, and stromelysin, all of which are capable of degrading collagen, elastin, and proteocoglycans.[23-27] Such processes could lead to thinning of the fibrous cap and disruption, particularly if potent mechanical stresses are present. We are currently investigating the contribution of macrophage infiltration and apoptosis to the relative activity of metalloproteinases and tissue inhibitors of metalloproteinases. Preliminary results indicate that the apoptotic rate is proportional to the number of infiltrating macrophages and is related to symptomatic disease and to the position of the necrotic core within the plaque. Macrophage infiltration and apoptosis may modulate lesion progression and necrosis by the release of injurious free radicals and other mitogenic and tissue necrosis factors.[28-30] Activated macrophages may also induce a prothrombotic effect by inhibiting tissue plasminogen activators and thereby enhancing the thrombotic complications associated with complex atherosclerotic plaques.[32,33]

Our results also indicate that plaque complications such as intraplaque hematoma and surface thrombosis, although characteristic of large advanced plaques, did not discriminate between symptomatic and asymptomatic plaques and appeared to represent events, probably as a result of plaque fissuring or rupture.

The potential role of biomechanical forces in inducing structural fatigue of plaque constituents and the localization of plaque neoformation and inflammatory cell responses is under study. Marked elevation of wall shear stress occurs within stenoses that are associated with large plaques. Although high

shear may inhibit plaque formation,[34] changes in flow dynamics associated with marked stenoses, including wall vibration, flutter, and cyclical collapse,[35] could induce disruptions within plaques, lumen ulcer formation, and associated surface irregularities. Vito and others[36,37] have emphasized the relationship between plaque composition and the location of peak stress. For example, it has been found that proximity of the necrotic core to the lumen increases the stress concentration in the overlying fibrous cap. Others have demonstrated an association between regions of macrophage infiltration and mechanical stress concentration.

The findings of this study suggest that a key associative event in carotid plaque fibrous cap disruption and symptoms are the reactions induced by macrophage or foam cell infiltration in the abluminal fibrous cap, as well as the proximity of the necrotic core to the lumen, which result in the thinning and eventual erosion of the fibrous cap. In vivo studies of the interactions between plaque composition and the corresponding flow and tensile-mechanical–associated properties are warranted, especially when morphologic and mechanical properties may be studied in these same plaques removed at endarterectomy. Such detailed correlations could help identify these lesions and mechanical factors that render plaques susceptible to disruption and to the onset of symptomatic carotid artery disease.

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