Histological Correlates of Carotid Plaque Surface Morphology on Lumen Contrast Imaging

J.K. Lovett, MB ChB; P.J. Gallagher, PhD; L.J. Hands, MS; J. Walton, BSc (Hons); P.M. Rothwell, PhD

Background—Carotid angiographic plaque surface morphology is a powerful risk factor for stroke and systemic vascular risk. However, the underlying pathology is unclear, and a better understanding is required both to evaluate other forms of carotid imaging and to develop new treatments. Previous studies comparing angiographic plaque surface morphology with pathology have been small and unblinded, and the vast majority assessed only the crude macroscopic appearance of the plaque. We performed the first large study comparing angiographic surface morphology with detailed histology.

Methods and Results—Carotid plaque surface morphology was classified as ulcerated, irregular, or smooth on 128 conventional selective carotid artery angiograms from consecutive patients undergoing endarterectomy for severe symptomatic stenosis. Blinded angiographic assessments were compared with 10 histological features recorded on detailed microscopy of the plaque using reproducible semiquantitative scales. Angiographic ulceration was associated with plaque rupture ($P=0.001$), intraplaque hemorrhage ($P=0.001$), large lipid core ($P=0.005$), less fibrous tissue ($P=0.003$), and increased instability overall ($P=0.001$). For example, angiographically ulcerated plaques were much more likely than smooth plaques to be ruptured (OR = 15.4, 95% CI = 2.7 to 87.3, $P<0.001$), show a large lipid core (OR = 26.7, 95% CI = 2.6 to 270, $P<0.001$) or a large hemorrhage (OR = 17.0, 95% CI = 2.0 to 147, $P=0.02$). The equivalent odds ratios for angiographically irregular versus smooth plaque were 6.3 (1.3 to 31, $P=0.02$), 6.7 (1.5 to 30, $P=0.008$), and 9.2 (1.1 to 77, $P=0.02$), respectively.

Conclusions—In contrast to previous studies based on macroscopic assessment, we found very strong associations between detailed histology and carotid angiographic plaque surface morphology. Plaque surface morphology on carotid angiography is a highly sensitive marker of plaque instability. Studies of the predictive value of MR- and CT-based lumen contrast plaque surface imaging are required. (Circulation. 2004;110:2190-2197.)

Key Words: carotid arteries | angiography | plaque

Studies of lumen contrast angiography have provided valuable prognostic information in the carotid and coronary circulations. An appearance of ulceration of a symptomatic carotid stenosis on intra-arterial contrast angiography is a strong independent predictor of stroke, and similar appearances on coronary angiography also have prognostic significance. Irregularity of carotid plaque on angiography has been shown to predict not only stroke but also acute coronary events on follow-up. Although the assessment of carotid plaque morphology on angiography is well described and reproducible, no definitive histological study has been performed to determine what it represents and why it is such a powerful predictor of ipsilateral stroke and systemic vascular risk. The pathological significance of coronary angiographic plaque surface morphology is even less clear, partly because coronary plaque is less readily available for study. The few data that do exist relate to comparisons with other imaging modalities or to angiography performed post mortem. Histological correlation with angiographic carotid plaque surface morphology is important because it is unclear whether less invasive forms of lumen contrast imaging, such as CT or MR angiography, have the same predictive power as conventional angiographic assessments, and long-term follow-up studies of these techniques in the carotid artery are not possible in symptomatic patients because endarterectomy is now indicated. However, conventional intra-arterial contrast carotid angiography is now seldom performed in unselected patients with recently symptomatic carotid stenosis, and so existing data on histological correlates of angiographic carotid plaque surface morphology are important. Unfortunately, the majority of studies that are referenced as comparisons of carotid angiographic plaque surface morphology with “pathology” have assessed only the gross macroscopic appearance of the plaque surface recorded by the surgeon at endarterectomy (Table 1). The surgeons were not generally blinded to the angiographic appearance.
and it is uncertain how these macroscopic assessments correlate with the underlying histology. To the best of our knowledge, there have been no large detailed studies comparing angiographic plaque surface morphology with histological appearance. Of 4 published studies that have reported some comparison with histological assessment, 2,21–24 2 provide only qualitative results, 23,24 and 2 reported only comparisons between angiographic ulceration and histological plaque rupture.21,22 We have therefore performed the largest study of this kind (128 carotid plaques) to determine which of 10 histological features are associated with assessments of plaque ulceration on angiography. We have also investigated the histological significance of plaque surface irregularity and ulcers of different types. We studied intra-arterial angiography because it is still considered to be the “gold standard” for carotid artery lumen imaging and because it has prognostic significance.1,2,5 If no association is found between conventional intra-arterial angiographic appearance and histology, it is unlikely that data obtained on lumen surface morphology with noninvasive techniques of lumen contrast imaging will provide any useful information about plaque stability.

Methods

Patients
We studied the carotid arteries of consecutive patients who had carotid angiography and who underwent carotid endarterectomy in Oxford. At the time of the study, it was policy to operate only on patients with symptomatic carotid stenosis of at least 70% by the method used in the European Carotid Surgery Trial (equivalent to 50% NASCET method) on angiography.25 Patients were excluded if they were undergoing surgery for restenosis or radiotherapy-induced carotid stenosis.

Histopathology
The excised plaque was fixed in formalin immediately after removal. The portion of the carotid bifurcation showing maximum disease was divided transversely (ie, perpendicular to the lumen) at 3-mm

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| TABLE 1. Previous Studies That Compared Angiographic Carotid Plaque Surface Morphology With Pathological Appearance |
|---|---|---|---|---|
| Studies of histological appearance | Studies of gross macroscopic appearance only |
| No. of Plaques | No. With Angiographic Ulceration (%) | Blinding Stated | Pathological Features | Sensitivity | Specificity |
| Estol, 1991 | 36 | 12 (33) | Yes | Rupture | 56% | 89% |
| Kim, 2000 | 55 | 46 (84) | Yes | Rupture | 86% | 33% |
| Croft, 1980 | 64 | 2 (3) | Yes | Rupture | Not given |
| Endo, 1996 | 40 | 6 (15) | No | Rupture, thrombus and hemorrhage | 4 of 6 ulcers were found in plaque with rupture, hemorrhage and localized thrombus |
| Maddison, 1969 | 45 | 38 (84) | No | Macroscopic ulceration | 88% | 0% |
| Houser, 1974 | 349 | 139 (40) | No | Macroscopic ulceration | Not given | 100% |
| Blaisdell, 1974 | 50 | 38 (76) | No | Macroscopic ulceration | 100% | 63% |
| Edwards, 1979 | 50 | 29 (58) | No | Macroscopic ulceration | 60% | 43% |
| O’Donnell, 1985 | 79 | 30 (38) | Yes | Macroscopic ulceration | 59% | 73% |
| Eikelboom, 1983 | 155 | 84 (54) | No | Macroscopic ulceration | 73% | 62% |
| Ricotta, 1986 | 84 | 54 (64) | Yes | Macroscopic ulceration | 78% | 56% |
| Comerota, 1990 | 126 | 44 (35) | No | Macroscopic ulceration | 53% | 92% |
| Streiff, 1994 | 480 | 181 (38) | No | Macroscopic ulceration | 46% | 74% |
| Kagawa, 1996 | 48 | 24 (50) | No | Macroscopic ulceration | 57% | 73% |
| Liberopoulos, 1996 | 52 | 25 (48) | small ulcers | 16 (30) | large ulcers | 93% small ulcers | Not given |
| Rothwell, 2000 | 1671 | 1066 (63.8) | No | Macroscopic thrombus | 69% (ulcer) | 47% (ulcer) |

Irregular or ulcerated
intervals for embedding in paraffin wax. Transverse sections 5 to 10 μm thick were taken from each wax block at 3-mm intervals along the length of the plaque. Four adjacent sections were taken from each block and stained with (1) hematoxylin and eosin (H&E), (2) elastin van Gieson to stain the fibrous cap, (3) CD68 antibody to stain macrophages, and (4) CD3 antibody to stain lymphocytes.

Histological Features
For each plaque, the presence and/or amount of the following features was recorded on a simple semiquantitative 3- or 4-grade scale (Table 2): rupture of the fibrous cap, lipid core size, nodular calcification, neovascularization, inflammatory infiltrate, infiltration of the fibrous cap, proportion of fibrous tissue, intraplaque hemorrhage, and surface thrombus (Figure 2).
Lipid core was defined as an area of amorphous material containing cholesterol crystals. A large lipid core was considered to be one that occupied at least 50% of the thickness of the plaque or one that occupied at least 25% of the total section area. Calcification was considered to be present in large amounts when nodular deposits were seen or small amounts when there was stippling only, as illustrated by Jeziorska et al. Intraplaque hemorrhage included recent or old hemorrhage as defined by Bassiouny et al. Rupture was recorded where there was clear communication between the lipid core and the lumen with a break in the fibrous cap, usually at a point of thinning and inflammation, and where the break in the cap did not seem to have been created during surgery. Plaque and cap inflammation was graded according to the number of CD68- or CD3-staining cells. Thrombus was recorded when there was an organized collection of fibrin and red blood cells in the lumen as illustrated by Lammie et al.

Minimum cap thickness was also measured using a calibrated graticule in the microscope eyepiece. A thin cap was defined as fibrous tissue interposed between the lumen and the lipid core, which had a minimum measured thickness of ≤200 μm or where there was complete rupture with no remaining cap visible. This value was used because it was the median minimum cap thickness recorded in our study.

Each plaque was also classified according to the American Heart Association (AHA) classification of coronary atherosclerosis. Because these specimens were all of advanced atherosclerotic lesions, the principle differentiation was made between “uncomplicated” atherosclerosis (AHA grades IV and V) and “complicated” atherosclerosis (AHA grade VI). AHA grade VI was defined as showing prominent rupture, hemorrhage or thrombus. However, the AHA classification does not take into account important features that determine plaque stability, such as cap thinning, lipid core size, or inflammation. Therefore, we also classified each plaque overall as being definitely stable, probably stable, probably unstable, or definitely unstable (Table 2). The aim of this classification was to take into account all potential markers of instability, and the definitions were based on widely accepted descriptions of unstable plaque in the coronary circulation.

An observer who was experienced in vascular pathology examined all the histology sections blinded to clinical characteristics and angiography. We have previously demonstrated that our histological assessments are reproducible (between and within observers) and that there is agreement between adjacent 3-mm sections. Our intraobserver k values were 0.76 (95% CI = 0.76 to 0.98) for the assessment of hemorrhage, 0.89 (95% CI = 0.76 to 1) for thrombus, 0.87 (95% CI = 0.76 to 0.98) for lipid core size, 0.65 (95% CI = 0.48 to 0.82) for foam cells, 0.75 (95% CI = 0.63 to 0.87) for new vessels, 0.80 (95% CI = 0.68 to 0.92) for calcification, 0.68 (95% CI = 0.52 to 0.84) for inflammation, 0.83 (95% CI = 0.73 to 0.93) for rupture, and 0.69 (95% CI = 0.51 to 0.87) for overall instability.

Statistical Analyses
We calculated the statistical significance of associations between the histological findings and angiographic plaque surface morphology. For those histological features that were significantly associated with plaque surface morphology, odds ratios for the presence of each feature were calculated for ulcerated versus smooth plaques on angiography. In addition to the overall analysis, separate analyses were performed for each ulcer type. Calculations were made using the statistical software SPSS for Windows (version 10.0).

Results
In all, 141 carotid plaques were collected at endarterectomy from patients with symptomatic severe carotid stenosis who had undergone intra-arterial carotid angiography preoperatively. Ten plaques (7%) were excluded from the study because the carotid specimen was fragmented, and 3 (2%) were excluded because there were insufficient angiographic views. Of the 128 remaining patients included in the study, 97 (76%) were male, 64 (50%) had treated hypertension, 69 (54%) were smokers, 13 (10%) had previously treated hyperlipidemia, 90 (70%) were taking antiplatelet therapy, and 8 (6%) had treated diabetes mellitus preoperatively. The mean age was 61.3 years (SD = 9.4). The median time between angiography and surgery was 35 days (interquartile range, 13 to 61 days).
Angiographic plaque surface morphology was significantly associated with the presence of rupture of the fibrous cap (significance for trend: \( P = 0.001 \)), intraplaque hemorrhage \( (P=0.001) \), lipid core size \( (P=0.005) \), and proportion of fibrous tissue \( (P=0.003) \) on histological examination (Figure 3). There were no significant associations with surface thrombus \( (P=0.1) \), fibrous thickness cap <200 \( \mu m \) \( (P=0.3) \), foam cells \( (P=0.4) \), new vessels \( (P=0.06) \), calcification \( (P=0.2) \), inflammation \( (P=0.08) \), or cap infiltration \( (0.2) \). The odds ratios for angiographically ulcerated versus smooth plaque were 15.4 (95% CI=2.7 to 87.3, \( P = 0.001 \)) for definite rupture versus definitely no rupture, 26.7 (95% CI=2.6 to 270.6, \( P<0.001 \)) for large lipid core versus no lipid core, and 17.0 (95% CI=2.0 to 147.2, \( P=0.002 \)) for large hemorrhage versus no hemorrhage. The equivalent odds ratios for angiographically irregular versus smooth plaque were 6.3 (95% CI=1.3 to 31.5, \( P=0.02 \)), 6.7 (95% CI=1.5 to 30.1, \( P=0.008 \)), and 9.2 (95% CI=1.1 to 77.3, \( P=0.02 \)), respectively.

Angiographic surface morphology was significantly associated with AHA grade (VI versus IV or V, \( P=0.01 \), Figure 3). However, the strongest association was with the assessment of overall plaque instability \( (P=0.001, \text{Figure 4}) \). The odds ratios for presence of a definitely unstable plaque versus

### Table 2. Semiquantitative Grading Scales Used to Assess Presence of Histological Features

<table>
<thead>
<tr>
<th>Histological Feature</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemorrhage</td>
<td>No hemorrhage</td>
<td>Small hemorrhage</td>
<td>Large hemorrhage</td>
<td>N/A</td>
</tr>
<tr>
<td>Thrombus</td>
<td>No thrombus</td>
<td>Small thrombus</td>
<td>Large thrombus</td>
<td>N/A</td>
</tr>
<tr>
<td>Lipid core</td>
<td>No lipid core</td>
<td>Small lipid core</td>
<td>Large lipid core (~&gt;25% total area)</td>
<td>N/A</td>
</tr>
<tr>
<td>Fibrous tissue</td>
<td>Very little fibrous tissue</td>
<td>~50% fibrous tissue</td>
<td>Predominantly fibrous</td>
<td>N/A</td>
</tr>
<tr>
<td>Foam cells</td>
<td>None</td>
<td>&lt;50 cells</td>
<td>At least 50 cells</td>
<td>N/A</td>
</tr>
<tr>
<td>New vessels</td>
<td>None</td>
<td>&lt;10 per section</td>
<td>At least 10 per section</td>
<td>N/A</td>
</tr>
<tr>
<td>Calcification</td>
<td>None</td>
<td>Stippling only</td>
<td>Calcified nodules</td>
<td>N/A</td>
</tr>
<tr>
<td>Inflammatory cells*</td>
<td>None</td>
<td>Occasional cells or one group of &gt;50 cells</td>
<td>&gt;5 groups of &gt;50 cells or one group &gt;500 cells</td>
<td>N/A</td>
</tr>
<tr>
<td>Cap infiltration*</td>
<td>None</td>
<td>&lt;10 cells in cap</td>
<td>10–50 cells in cap</td>
<td>&gt;50 cells in cap</td>
</tr>
<tr>
<td>Rupture</td>
<td>Intact cap</td>
<td>Probably intact, eg, artefactual break in cap from surgical incision</td>
<td>Probably ruptured, eg, site of rupture not clear but thrombus seen adherent to lipid in lumen</td>
<td>Definitely ruptured</td>
</tr>
<tr>
<td>Overall instability</td>
<td>Definitely stable, eg, predominantly fibrous, few inflammatory cells, intact cap</td>
<td>Probably stable, eg, one feature of instability such as small hemorrhage or inflamed</td>
<td>Probably unstable, eg, inflammation, thin cap, and large core but no rupture</td>
<td>Definitely unstable, eg, rupture, thrombus, large hemorrhage, thin inflamed cap</td>
</tr>
</tbody>
</table>

*Macrophages or lymphocytes stained respectively with CD68 or CD3 antibody.

![Figure 3](https://circ.ahajournals.org/figure3.png) Relative frequencies of plaques with ulcerated, irregular, or smooth surface on carotid angiography (y axis) by grade of histological feature (x axis): rupture, hemorrhage, lipid core size, and proportion of fibrous tissue. Probability values are given for significance of trend. Semiquantitative scales used to classify histological features are described in Table 2.
a definitely stable plaque were 14.4 (95% CI=2.8 to 73.0, \(P<0.001\)) for ulcerated versus smooth plaque on angiography and 4.9 (95% CI=1.2 to 20.8, \(P=0.02\)) and for irregular versus smooth plaque. The associations were not significantly different in patients who were taking antiplatelet therapy versus no antiplatelet therapy or in patients whose time from angiography to surgery was <1 month versus >1 month.

For the categorization of ulcer type, the intraobserver and interobserver agreements were good: 79% (\(\kappa=0.70, \text{95\% CI}=0.58\) to 0.81) and 74% (\(\kappa=0.62, \text{95\% CI}=0.49\) to 0.74), respectively. Although the numbers of each ulcer type were small, significant associations were found between the presence of each of the 4 ulcer types (versus smooth plaques) and rupture, hemorrhage, fibrous tissue, and overall instability (Table 3). Lipid core size was associated with types 1 and 2, but there was no significant association with types 3 and 4.

**Discussion**

We have shown that plaque surface morphology on angiography is strongly associated with the presence of rupture, intraobserver plaque hemorrhage, lipid core size, and proportion of fibrous tissue in carotid plaque. Furthermore, angiographic appearances of ulceration and irregularity are strong predictors of overall carotid plaque instability when it is assessed in a similar way to instability described in coronary pathological studies.\(^{30-34}\) Our results complement studies of coronary atheroma that show that the appearance of a complex, ulcerated plaque on coronary angiography is associated with adverse coronary outcomes on follow-up\(^1\) and the appearance of rupture on intravascular ultrasound.\(^36\) However, this appearance on coronary angiography has been difficult to validate pathologically because coronary specimens are not so readily accessible for in vivo comparisons as carotid plaques, and data are usually available only for ex vivo angiography after death.\(^8\)

Our results provide important information on the mechanism of symptomatic carotid artery disease. In the coronary circulation, the pathology of unstable plaque has been well described, and pathological studies suggest that plaque morphology is more important than degree of stenosis as a cause of acute thrombotic events.\(^{30,32-34,37}\) However, in the carotid circulation, severity of stenosis is also important,\(^1,2\) and hypoperfusion may have a more significant role in the production of symptoms.\(^{38}\) Nevertheless, large angiographic studies have shown that plaque surface morphology predicts risk of ipsilateral ischemic stroke independent of stenosis and vascular risk factors,\(^1,2\) but there have been no definitive studies to determine exactly what angiographic plaque surface morphology is measuring and why it is a powerful predictor of stroke and also systemic vascular risk.\(^5\) Our study is the largest study ever to compare angiographic surface morphology with plaque histology. Moreover, it is the first to assess the significance of irregularity and ulcer shape on angiography and to compare angiographic appearance with a

**TABLE 3. Trend Associations Between Types of Ulcer (47 Ulcers vs 17 Smooth Plaques) and Histological Characteristics (2–4 Grade Scales)**

<table>
<thead>
<tr>
<th>Histological Characteristic</th>
<th>Type 1 Ulcer (n=18)</th>
<th>Type 2 Ulcer (n=10)</th>
<th>Type 3 Ulcer (n=13)</th>
<th>Type 4 Ulcer (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rupture</td>
<td>0.010</td>
<td>0.003</td>
<td>0.021</td>
<td>0.011</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>0.005</td>
<td>&lt;0.001</td>
<td>0.025</td>
<td>0.042</td>
</tr>
<tr>
<td>Lipid core size</td>
<td>0.020</td>
<td>0.018</td>
<td>0.064</td>
<td>0.107</td>
</tr>
<tr>
<td>Fibrous tissue</td>
<td>0.001</td>
<td>0.004</td>
<td>0.012</td>
<td>0.003</td>
</tr>
<tr>
<td>Overall instability</td>
<td>0.020</td>
<td>0.003</td>
<td>0.020</td>
<td>0.031</td>
</tr>
</tbody>
</table>
detailed histological assessment that included quantification of 10 separate histological features. Our findings that angiographic irregularity or ulceration predict rupture, intraobserver plaque hemorrhage, large lipid core, and reduced fibrous tissue support the hypothesis that these features are as important in carotid plaque instability as they are in coronary plaque instability.

Of particular interest is our finding that intraobserver plaque hemorrhage is at least as strongly associated with angiographic ulceration as plaque rupture. It is likely that plaque rupture allows blood and contrast from the lumen into the necrotic core, which gives the appearance of an ulcer on angiography. Nevertheless, descriptions of coronary plaques suggest that intraobserver plaque hemorrhage also arises by an alternate mechanism: bleeding from the small vessels within the plaque. 19,31 However, it is less likely that hemorrhage arising from capillaries would be associated with angiographic ulceration.

Interestingly, we found that thrombus was not significantly associated with angiographic plaque surface morphology. It is possible that, if thrombus forms over the site of rupture, contrast cannot enter the plaque and angiographic ulceration is not seen. Another possibility is that thrombus can disappear between the date of angiography and date of surgery, or become detached at the time of surgery. However, the relationship between angiographic appearance and thrombus was not stronger in patients who had surgery within 1 month or in patients who were not on antplatelet therapy.

Limitations of Our Study
To study a large number of carotid plaques, we chose to use a relatively simple technique for the histological assessment, and we took sections at 3-mm intervals along the length of the diseased portion of the bifurcation. It is therefore inevitable that histological features that were present only to a small extent and that were situated between the sites of sectioning were missed. However, we have previously demonstrated that our histological assessments are reproducible, that there is good agreement between adjacent 3-mm sections, and that even single bifurcation sections are fairly representative of the plaque histology as a whole. 35 Moreover, any inaccuracies introduced by our histology method would tend to reduce the size and significance of any associations that we have found. This suggests that the true associations between carotid angiography and histology may, in fact, be stronger.

A second potential limitation of our study is that all the carotid plaques included in our study were from symptomatic patients. Therefore, although it seems likely that the associations between angiographic plaque surface morphology and plaque histology would also be present in asymptomatic patients, our results are directly applicable only to symptomatic patients.

Conclusions
In a large number of plaques, we have shown that the appearance of ulceration or irregularity seen on carotid lumen imaging is associated with the presence of cap rupture, intraobserver plaque hemorrhage, a large lipid core, reduced fibrous tissue, and plaque instability similar to that found in the coronary circulation. This confirms the hypothesis generated from our previous observation that angiographic carotid plaque surface morphology predicts acute coronary events, ie, that the pathological process in the carotid and coronary arteries is similar. 6 Our data highlight the potential of carotid lumen contrast imaging as a method of identification of patients at high coronary risk, but further studies of MR- or CT-based lumen contrast plaque surface imaging are required.

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References


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