Histological Features of Carotid Plaque in Patients With Ocular Ischemia Versus Cerebral Events

Dominic P.J. Howard, MA, MRCS*; Guus W. van Lammeren, PhD*; Jessica N. Redgrave, DPhil; Frans L. Moll, PhD; Jean-Paul P.M. de Vries, PhD; Dominique P.V. de Kleijn, PhD; Gert Jan de Borst, PhD; Gerard Pasterkamp, PhD; Peter M. Rothwell, PhD

Background and Purpose—Patients with carotid artery stenosis and ocular ischemic events have a much lower risk of future ipsilateral ischemic stroke on medical treatment and lower procedural risks for endarterectomy and stenting than patients with cerebral ischemic events, and are closer in risk to patients with asymptomatic stenosis. The reasons for this difference in prognosis are not fully understood, but may reflect differences in carotid plaque pathology.

Methods—In consecutive patients undergoing carotid endarterectomy for recently symptomatic stenosis (Oxford Plaque Study, Athero-Express Study), we compared carotid plaque histology (using validated semiquantitative scales) in those who had cerebral events within the last 6 months (n=1317) versus those with ocular events only (n=323).

Results—Compared with plaques from patients with ocular events only, those from patients with cerebral events had significantly more large lipid core (odds ratio [OR], 1.38; 95% confidence interval [CI], 1.05–1.82; P=0.02), inflammation (OR, 1.32; 95% CI, 1.02–1.72; P=0.04) and overall plaque instability (OR, 1.37; 95% CI, 1.05–1.80; P=0.02), and less fibrous content (OR, 0.71; 95% CI, 0.54–0.92; P=0.01), and calcification (OR, 0.70; 95% CI, 0.54–0.91; P=0.008). The overall number of histological features known to be associated with vulnerable plaque was greater in patients with cerebral events than in those with ocular events (P=0.002).

Conclusions—Carotid plaques from patients undergoing endarterectomy for previous ocular ischemic events have fewer vulnerable plaque features than those from patients with recent cerebral ischemic events, possibly explaining some of the differences in risk of stroke between these groups. (Stroke. 2013;44:734-739.)

Key Words: acute stroke ■ amaurosis fugax ■ carotid atherosclerotic plaque ■ ocular ischemia

The risk of ipsilateral ischemic stroke in patients with recent ocular ischemic events is less than half that of otherwise similar patients with recent cerebral ischemic events, and in those with carotid stenosis, their prognosis is similar to that in patients with asymptomatic stenosis. Consequently, in patients with ≥70% carotid artery stenosis in the European Carotid Surgery Trial and North American Symptomatic Carotid Endarterectomy Trial (NASCET), the absolute reduction in 5-year risk of ipsilateral ischemic stroke with surgery was 5% for patients with recent ocular events and 17% for those with cerebral events, despite a lower procedural risk in the patients with ocular events only. Across all carotid endarterectomy trials and case-series, the 30-day risk of stroke in patients with ocular ischemic events is less than half that in patients with cerebral events independent of degree of carotid artery stenosis, and the same difference has been observed in the procedural risk of carotid stenting.

The reasons for the relatively low risk of ipsilateral ischemic stroke in patients with carotid stenosis and ocular ischemic events are not fully understood. However, the fact that these patient groups have the same prevalence of previous coronary heart disease and peripheral vascular disease and the same risk of future coronary events suggests that the lower stroke risk in patients with ocular events only may reflect differences in local carotid plaque pathology. The only previous study comparing histological plaque features from patients with ocular versus cerebral ischemic events suggested that patients with ocular events may have more stable plaques, but the number of cases was too small to allow reliable adjustment for differences in other risk factors. We aimed to study carotid plaque composition in a larger number of consecutive patients undergoing carotid endarterectomy for symptomatic stenosis to compare features in those who had cerebral events within the last 6 months versus those with ocular events only.
Methods

We studied data from 2 large carotid plaque biobanks in the United Kingdom (Oxford Plaque Study) and the Netherlands (Athero-Express Study) in which plaques from consecutive patients undergoing carotid endarterectomy underwent detailed histological assessment with reproducible semiquantitative scales. Patients were included if they had a symptomatic ischemic event within 6 months before carotid endarterectomy. All consecutive consenting patients who underwent endarterectomy were included. The Oxford Plaque Study included 481 plaques collected between 1975 and 2002, and the Athero-Express Study included 1159 plaques collected between 2002 and 2011 (total n=1640). All patients and their relevant imaging were reviewed by a neuropathologist before carotid endarterectomy. Patients were categorized as having only ocular ischemic events or cerebral events during the 6 months before endarterectomy. Ocular symptoms were defined as amaurosis fugax or retinal infarction without symptoms of transient or sustained cerebral ischemia. Patients were excluded if they had undergone surgery for asymptomatic disease, restenosis, or radiolysis-induced carotid stenosis. Patients were operated under local anesthesia or general anesthesia with selective shunt use based on either clinical status (for local anesthesia) or standard electroencephalogram or transcranial Doppler criteria (for general anesthesia). Endarterectomies were performed by either conventional or eversion techniques, with careful dissection of the bifurcation into the internal and external carotid arteries. Atherosclerotic plaque was harvested and transported to the laboratory immediately after dissection.

The processing and assessment of carotid plaques has been described in detail for both studies previously.\textsuperscript{12–15} Briefly, carotid plaques were formalin-fixed after endarterectomy, and either the whole plaque (Oxford Plaque Study) or the portion of the plaque showing maximum disease (Athero-Express Study) was paraffin-embedded. After creating transverse sections, plaques were immunohistochemically stained with (1) hematoxylin and eosin (H&E) for assessment of overall plaque stability, lipid core, thrombus, and intra-plaque hemorrhage, (2) elastin von Gieson for fibrous plaque content, and (3) CD68 for plaque macrophage content. Both studies applied comparable 3-, or 4-point semiquantitative scales for the assessment of overall plaque stability, lipid core, fibrous plaque content, microvessel density (neovascularization), macrophage infiltration, and calcifications, as defined previously (Table 1 in the online-only Data Supplement).\textsuperscript{12–15} To maximize comparability and overlap of definitions between studies, the scales were transformed into binominal variables (Table I in the online-only Data Supplement).

Overall plaque instability was based on a modified American Heart Association classification used for coronary atherosclerosis, which incorporates important features that determine carotid plaque stability, such as lipid core size, inflammation, and fibrous content, as described previously.\textsuperscript{15} The concept of plaque vulnerability has been well described for coronary atherosclerotic lesions,\textsuperscript{16} and more recently has been applied to the carotid circulation.\textsuperscript{17,18} We have assessed for the presence of multiple plaque characteristics thought to be associated with vulnerability (intraplaque hemorrhage, thrombus, large lipid core, low fibrous content, and inflammation-macrophage infiltration) to grade the degree of plaque vulnerability.

SPSS version 18.0 (Chicago, IL) was used for all statistical analyses. Baseline demographics for ocular and cerebral event patients were examined with Student t test and Mann-Whitney U test for parametric and nonparametric continuous variables, respectively. Baseline differences in nominal variables were examined with Pearson χ² test. Associations between binominal plaque characteristics and presenting symptoms were assessed with adjusted odds ratios (ORs) produced from multivariable binary logistic regression analysis, including potentially confounding baseline differences (age, sex, diabetes mellitus, hypercholesterolemia, and hypertension). Logistic regression analysis was also used to test for differences in the presence of multiple vulnerable plaque characteristics in relation to clinical presentation. Heterogeneity between study cohorts for individual plaque features was assessed for using interaction terms in binary logistic regression. Because no significant heterogeneity was found, binominal data from both studies were combined for pooled analysis. However, all such analyses were still stratified by study cohort. Three-year follow-up data were available for 99.0% (1624/1640) of patients. Outcome event rates were derived by Kaplan–Meier analysis.

Results

The Oxford Plaque Study comprised 481 plaques, and the Athero-Express Study comprised 1159 plaques, resulting in a total sample of 1640 plaques (323 with ocular ischemic events only and 1317 with cerebral ischemic events). The baseline characteristics and differences between patients stratified by presenting event and study are provided in Table 1. In the Oxford Plaque Study, patients with cerebral events had higher prevalence of diabetes mellitus, treated hypercholesterolemia (statin therapy), and hypertension compared with those with ocular events. In the Athero-Express Study, patients with cerebral events were older, and in the total pooled sample, patients with cerebral symptoms were older and had a higher prevalence of diabetes mellitus, treated hypercholesterolemia, and hypertension than those with ocular events. We, therefore, provide ORs both unadjusted and adjusted for age, sex, diabetes mellitus, and treated hypercholesterolemia and hypertension in our analyses. In neither cohort was there any difference between patients with ocular events only and those with cerebral events in the frequency of previous myocardial infarction, peripheral vascular disease, or degree of symptomatic carotid stenosis. During 3-year follow-up postendarterectomy, rates of myocardial infarction were similar in both groups (Figure).

Within the cerebral events group, 39.6% of patients were classified as having partial anterior circulation strokes, 54.4% as nonlacunar transient ischemic attacks, and 6.0% as possible lacunar events. Within the ocular group, 80.8% were amaurosis fugax, and 19.2% were retinal artery occlusions. For 93.8% of patients, this event was their first-ever event ipsilateral to the side of carotid stenosis; 69.2% of patients in the ocular group and 60.4% in the cerebral group had multiple events within the 6-month period before surgery.

Table 2 shows the histological characteristics of plaques in patients with ocular events only versus those with cerebral events analyzed separately in each study. The findings were similar in the 2 studies, with no statistically significant heterogeneity for any characteristic. In the pooled analysis, plaques from cerebral event patients were more unstable overall (OR, 1.39; 95% confidence interval [CI], 1.07–1.79; P=0.01), had greater macrophage staining (OR, 1.33; 95% CI, 1.04–1.71; P=0.03), were more likely to have a large lipid core (OR, 1.38; 95% CI, 1.06–1.80; P=0.02) and lower fibrous content (OR, 0.70; 95% CI, 0.54–0.90; P=0.006), and were less calcified (OR, 0.77; 95% CI, 0.60–0.98; P=0.03). Each of these differences remained statistically significant after adjustment for age, sex, diabetes mellitus, and treated hypercholesterolemia and hypertension; Table 2).

Analysis of additional plaque characteristics from each study, which were not overlapping, showed that lymphocyte infiltration (CD3 staining) was also significantly associated with cerebral events (Table 3). No other nonoverlapping characteristics were associated with presenting symptomatic event (Table 3). Plaque rupture tended to be more common...
in patients with cerebral events in the Oxford Plaque Study, but this did not reach statistical significance. Plaque rupture was not recorded in the Athero-Express Study. Additional subgroup analysis within the ocular event patient group revealed no significant differences in plaque composition for single amaurosis fugax versus multiple ocular symptoms versus retinal artery occlusions (data not shown). The median time from event to intervention was 56 days; 33.7% of patients were operated within 30 days of event. Subgroup analysis revealed that plaques removed within 30 days tended to be more unstable and inflammatory for both groups of patients (cerebral and ocular ischemic events), but the relative differences in the presence of plaque features remained fairly static (data not shown).

Analysis of the overall number of plaque characteristics believed to be associated with the vulnerable atherosclerotic plaque (presence of thrombus, large lipid core, low fibrous content, intraplaque hemorrhage, and macrophage infiltration) showed that the plaques from patients with cerebral event plaques had a significantly greater number of vulnerable plaque features than those from patients with ocular events only (Table 4; trend $P=0.002$).

**Discussion**

In the largest-ever study of carotid plaque histology, we have shown that plaques from patients with recent cerebral ischemic events differ significantly from those with recent ocular ischemic events. Plaques from patients with cerebral events were significantly more unstable, lipid-rich, and inflammatory, whereas those from patients with ocular events were significantly more fibrous and calcified. In addition, the overall number of vulnerable plaque features was greater in the plaques from patients with cerebral events when compared with those with ocular events.

These differences in local plaque histology may partly explain why the nature of the presenting symptoms differ (ie, ocular ischemia versus cerebral ischemia) and why the risk

---

**Table 1. Baseline Demographics From Patients With Monocular and Cerebral Symptoms in Oxford Plaque Study, Athero-Express Study, and Pooled Sample**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Oxford Plaque Study (n=481)</th>
<th>Athero-Express Study (n=1356)</th>
<th>Pooled Sample (n=1640)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Monocular (n=130)</td>
<td>Cerebral (n=351)</td>
<td>$P$ Value</td>
</tr>
<tr>
<td>Age, y (±SD)</td>
<td>66.5 (±7.4)</td>
<td>67.2 (±9.1)</td>
<td>0.43</td>
</tr>
<tr>
<td>Male</td>
<td>73.1% (95/130)</td>
<td>71.5% (251/351)</td>
<td>0.73</td>
</tr>
<tr>
<td>Median time since last event, days (IQ range)</td>
<td>90 (34–131)</td>
<td>87 (34–155)</td>
<td>0.57</td>
</tr>
<tr>
<td>Degree of symptomatic stenosis, mean (±SD)</td>
<td>83.6 (±11.7)</td>
<td>83.7 (±11.5)</td>
<td>0.90</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>6.2% (8/130)</td>
<td>12.5% (44/351)</td>
<td>0.05</td>
</tr>
<tr>
<td>Treated hypertension</td>
<td>49.2% (64/130)</td>
<td>61.5% (216/351)</td>
<td>0.02</td>
</tr>
<tr>
<td>Treated hypercholesterolemia*</td>
<td>6.9% (9/130)</td>
<td>21.9% (77/351)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Previous myocardial infarction</td>
<td>13.8% (18/130)</td>
<td>11.4% (40/351)</td>
<td>0.46</td>
</tr>
<tr>
<td>Peripheral vascular disease</td>
<td>22.3% (29/130)</td>
<td>15.7% (55/351)</td>
<td>0.09</td>
</tr>
</tbody>
</table>

IQ indicates interquartile.

*Previous statin therapy.
of ipsilateral ischemic stroke is lower in patients with ocular events despite similar prevalence of coronary artery disease and peripheral vascular disease and similar risks of future acute coronary events, as shown in our follow-up analysis. For example, it has been shown that different plaque types produce microembolic debris of different sizes and composition.19 Calcified, fibrous plaques, which we found to be associated with ocular events, tend to produce smaller, fibrin-rich emboli, whereas atheromatous, inflammatory plaques, which we have found to be more associated with cerebral events, release larger lipid-rich emboli.19 The vascular anatomy and potential collateral perfusion of the retina clearly differ from that of the brain, and it is likely to be less tolerant of small emboli. Although we lack the ability to routinely monitor asymptomatic

Table 2. Odds Ratios for Cerebral Symptoms Versus Monocular Symptoms, if Plaque Characteristics Are Present in Plaques From Oxford Plaque Study, Athero-Express Study, and Pooled Sample

<table>
<thead>
<tr>
<th>Study</th>
<th>Cerebral, n (%)</th>
<th>Ocular, n (%)</th>
<th>OR</th>
<th>95% CI</th>
<th>P Value</th>
<th>Adjusted OR*</th>
<th>95% CI</th>
<th>Adjusted P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall plaque instability</td>
<td>899/1310 (68.6)</td>
<td>194/319 (60.8)</td>
<td>1.39</td>
<td>1.07–1.79</td>
<td>0.01</td>
<td>1.37</td>
<td>1.05–1.80</td>
<td>0.02</td>
</tr>
<tr>
<td>Large lipid core</td>
<td>966/1312 (73.6)</td>
<td>209/322 (64.9)</td>
<td>1.38</td>
<td>1.06–1.80</td>
<td>0.02</td>
<td>1.38</td>
<td>1.05–1.82</td>
<td>0.02</td>
</tr>
<tr>
<td>Heavy macrophage staining</td>
<td>806/1309 (61.6)</td>
<td>177/318 (55.7)</td>
<td>1.33</td>
<td>1.04–1.71</td>
<td>0.03</td>
<td>1.32</td>
<td>1.02–1.72</td>
<td>0.04</td>
</tr>
<tr>
<td>Plaque hemorrhage</td>
<td>323/1316 (24.5)</td>
<td>79/323 (24.5)</td>
<td>1.12</td>
<td>0.84–1.49</td>
<td>0.45</td>
<td>1.10</td>
<td>0.81–1.48</td>
<td>0.54</td>
</tr>
<tr>
<td>High microvessel density</td>
<td>296/892 (33.4)</td>
<td>71/244 (29.1)</td>
<td>1.27</td>
<td>0.93–1.73</td>
<td>0.14</td>
<td>1.23</td>
<td>0.90–1.88</td>
<td>0.11</td>
</tr>
<tr>
<td>Thrombus</td>
<td>534/1314 (40.6)</td>
<td>118/322 (36.6)</td>
<td>1.12</td>
<td>0.87–1.45</td>
<td>0.38</td>
<td>1.09</td>
<td>0.84–1.42</td>
<td>0.52</td>
</tr>
<tr>
<td>Fibrous plaque</td>
<td>404/1317 (30.7)</td>
<td>125/323 (38.7)</td>
<td>0.70</td>
<td>0.54–0.90</td>
<td>0.006</td>
<td>0.71</td>
<td>0.54–0.92</td>
<td>0.01</td>
</tr>
<tr>
<td>Heavy calcification</td>
<td>655/1315 (49.8)</td>
<td>181/323 (56.0)</td>
<td>0.77</td>
<td>0.60–0.98</td>
<td>0.03</td>
<td>0.70</td>
<td>0.54–0.91</td>
<td>0.008</td>
</tr>
<tr>
<td>Oxford analyses (n=481)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall plaque instability</td>
<td>228/345 (66.1)</td>
<td>77/126 (61.1)</td>
<td>1.24</td>
<td>0.81–1.89</td>
<td>0.32</td>
<td>1.24</td>
<td>0.80–1.92</td>
<td>0.35</td>
</tr>
<tr>
<td>Large lipid core</td>
<td>222/351 (63.2)</td>
<td>75/130 (57.7)</td>
<td>1.26</td>
<td>0.84–1.90</td>
<td>0.27</td>
<td>1.28</td>
<td>0.84–1.96</td>
<td>0.26</td>
</tr>
<tr>
<td>Heavy macrophage staining</td>
<td>233/347 (67.1)</td>
<td>76/127 (59.8)</td>
<td>1.37</td>
<td>0.90–2.09</td>
<td>0.14</td>
<td>1.37</td>
<td>0.88–2.12</td>
<td>0.16</td>
</tr>
<tr>
<td>Plaque hemorrhage</td>
<td>122/351 (34.8)</td>
<td>44/130 (33.8)</td>
<td>1.04</td>
<td>0.68–1.59</td>
<td>0.85</td>
<td>0.97</td>
<td>0.62–1.50</td>
<td>0.88</td>
</tr>
<tr>
<td>High microvessel density</td>
<td>128/349 (36.7)</td>
<td>41/130 (31.5)</td>
<td>1.26</td>
<td>0.82–1.93</td>
<td>0.30</td>
<td>1.20</td>
<td>0.77–1.86</td>
<td>0.42</td>
</tr>
<tr>
<td>Thrombus</td>
<td>119/349 (34.1)</td>
<td>38/129 (29.5)</td>
<td>1.24</td>
<td>0.80–1.92</td>
<td>0.34</td>
<td>1.30</td>
<td>0.83–2.03</td>
<td>0.26</td>
</tr>
<tr>
<td>Fibrous plaque</td>
<td>108/351 (30.8)</td>
<td>49/130 (37.7)</td>
<td>0.74</td>
<td>0.48–1.12</td>
<td>0.15</td>
<td>0.70</td>
<td>0.45–1.08</td>
<td>0.11</td>
</tr>
<tr>
<td>Heavy calcification</td>
<td>169/351 (48.1)</td>
<td>67/130 (51.5)</td>
<td>0.87</td>
<td>0.58–1.31</td>
<td>0.51</td>
<td>0.83</td>
<td>0.54–1.27</td>
<td>0.39</td>
</tr>
<tr>
<td>Athero-Express analyses (n=1159)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall plaque instability</td>
<td>671/965 (69.5)</td>
<td>117/193 (60.6)</td>
<td>1.48</td>
<td>1.07–2.03</td>
<td>0.02</td>
<td>1.44</td>
<td>1.02–2.01</td>
<td>0.04</td>
</tr>
<tr>
<td>Large lipid core</td>
<td>744/961 (77.4)</td>
<td>134/192 (68.9)</td>
<td>1.50</td>
<td>1.06–2.11</td>
<td>0.02</td>
<td>1.47</td>
<td>1.02–2.12</td>
<td>0.04</td>
</tr>
<tr>
<td>Heavy macrophage staining</td>
<td>573/962 (59.6)</td>
<td>101/191 (52.9)</td>
<td>1.31</td>
<td>0.96–1.79</td>
<td>0.09</td>
<td>1.30</td>
<td>0.94–1.80</td>
<td>0.12</td>
</tr>
<tr>
<td>Plaque hemorrhage</td>
<td>201/965 (20.8)</td>
<td>35/193 (18.1)</td>
<td>1.19</td>
<td>0.80–1.77</td>
<td>0.40</td>
<td>1.22</td>
<td>0.80–1.86</td>
<td>0.36</td>
</tr>
<tr>
<td>High microvessel density</td>
<td>170/543 (31.3)</td>
<td>30/114 (26.3)</td>
<td>1.28</td>
<td>0.81–2.01</td>
<td>0.29</td>
<td>1.31</td>
<td>0.82–2.08</td>
<td>0.26</td>
</tr>
<tr>
<td>Thrombus</td>
<td>415/965 (43.0)</td>
<td>80/193 (41.5)</td>
<td>1.06</td>
<td>0.78–1.45</td>
<td>0.71</td>
<td>0.99</td>
<td>0.71–1.37</td>
<td>0.94</td>
</tr>
<tr>
<td>Fibrous plaque</td>
<td>296/966 (30.6)</td>
<td>76/193 (39.4)</td>
<td>0.68</td>
<td>0.50–0.94</td>
<td>0.02</td>
<td>0.70</td>
<td>0.50–0.98</td>
<td>0.04</td>
</tr>
<tr>
<td>Heavy calcification</td>
<td>486/964 (50.4)</td>
<td>114/193 (59.1)</td>
<td>0.71</td>
<td>0.52–0.96</td>
<td>0.03</td>
<td>0.64</td>
<td>0.46–0.89</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*Adjusted for age, sex, diabetes mellitus, treated hypercholesterolemia, and hypertension.

Table 3. Odds Ratios for Cerebral Symptoms Versus Monocular Symptoms for Nonoverlapping Plaque Characteristics

<table>
<thead>
<tr>
<th>Study</th>
<th>OR</th>
<th>95% CI</th>
<th>P Value</th>
<th>Adjusted OR*</th>
<th>95% CI</th>
<th>Adjusted P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxford Plaque Study (n=481)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plate rupture</td>
<td>1.45</td>
<td>0.96–2.18</td>
<td>0.08</td>
<td>1.43</td>
<td>0.93–2.18</td>
<td>0.10</td>
</tr>
<tr>
<td>Foam cells</td>
<td>1.13</td>
<td>0.75–1.70</td>
<td>0.56</td>
<td>1.16</td>
<td>0.76–1.78</td>
<td>0.49</td>
</tr>
<tr>
<td>Heavy lymphocyte staining</td>
<td>1.63</td>
<td>1.07–2.47</td>
<td>0.02</td>
<td>1.62</td>
<td>1.05–2.48</td>
<td>0.03</td>
</tr>
<tr>
<td>Heavy cap macrophage staining</td>
<td>1.14</td>
<td>0.70–1.87</td>
<td>0.60</td>
<td>1.02</td>
<td>0.61–1.72</td>
<td>0.94</td>
</tr>
<tr>
<td>Thin cap (&lt;200 µm)</td>
<td>1.12</td>
<td>0.70–1.80</td>
<td>0.64</td>
<td>1.08</td>
<td>0.66–1.77</td>
<td>0.76</td>
</tr>
<tr>
<td>Athero-Express Study (n=1159)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smooth muscle cells</td>
<td>0.76</td>
<td>0.54–1.08</td>
<td>0.13</td>
<td>0.80</td>
<td>0.56–1.16</td>
<td>0.24</td>
</tr>
</tbody>
</table>

CI indicates confidence interval; NA, not available; and OR, odds ratio.

*Adjusted for age, sex, diabetes mellitus, treated hypercholesterolemia, and hypertension.
Table 4. Odds Ratios for Presence of Multiple Vulnerable Plaque Characteristics (vs Zero Features) in Relation to Clinical Presentation in Pooled Sample: Cerebral Symptoms (n=1317) Versus Monocular Symptoms (n=323)

<table>
<thead>
<tr>
<th>Plaque characteristics</th>
<th>Cerebral, n (%)</th>
<th>Ocular, n (%)</th>
<th>OR</th>
<th>95% CI</th>
<th>P Value</th>
<th>Adjusted OR*</th>
<th>95% CI</th>
<th>Adjusted P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 vulnerable plaque features</td>
<td>128 (9.7)</td>
<td>49 (15.2)</td>
<td>1.00</td>
<td></td>
<td></td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 vulnerable plaque feature</td>
<td>174 (13.2)</td>
<td>50 (15.5)</td>
<td>1.23</td>
<td>0.78–1.95</td>
<td>0.38</td>
<td>1.21</td>
<td>0.75–1.95</td>
<td>0.44</td>
</tr>
<tr>
<td>2 vulnerable plaque features</td>
<td>204 (15.5)</td>
<td>55 (17.0)</td>
<td>1.33</td>
<td>0.85–2.08</td>
<td>0.22</td>
<td>1.25</td>
<td>0.79–1.99</td>
<td>0.34</td>
</tr>
<tr>
<td>3 vulnerable plaque features</td>
<td>380 (28.9)</td>
<td>79 (24.5)</td>
<td>1.68</td>
<td>1.11–2.54</td>
<td>0.01</td>
<td>1.65</td>
<td>1.07–2.54</td>
<td>0.02</td>
</tr>
<tr>
<td>≥4 vulnerable plaque features</td>
<td>431 (32.7)</td>
<td>90 (27.9)</td>
<td>1.69</td>
<td>1.13–2.53</td>
<td>0.01</td>
<td>1.66</td>
<td>1.08–2.54</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Data stratified by study group. χ² Linear-by-Linear association trend: P=0.002. CI indicates confidence interval; and OR, odds ratio.

*Adjusted for age, sex, diabetes mellitus, treated hypercholesterolemia, and hypertension.

retinal artery emboli, recent studies of middle cerebral artery microembolism during carotid artery intervention show that the brain is remarkably tolerant to small emboli, with plaques often shedding hundreds of emboli during interventions without any cerebral symptoms. However, there are also likely to be differences in cellular tolerance to transient ischemia and in the threshold for symptomatic awareness of a given level of ischemia between the retina and the brain.

The differences in plaque composition between patients with ocular versus cerebral events might also explain the lower procedural risks of stroke in patients with ocular events, both for endarterectomy and for stenting. In the case of carotid stenting, plaque composition has been associated with risk of periprocedural embolic events and early ipsilateral stroke. In the case of endarterectomy, MRI-detected ulcerated, irregular plaque surface has been reported to be associated with increased rates of periprocedural embolization.

We do not have detailed plaque imaging (echo-Doppler or MRI) for the majority of patients in this study. However, we have previously shown that plaque ulceration on angiography was strongly associated with several plaque features on histology. For the subset of patients in this study with angiographic carotid imaging (n=89), irregular or ulcerated plaque surface was seen in 64.5% of patients with ocular symptoms versus 70.7% with cerebral symptoms (P=0.55). Similarly, on macroscopic visual inspection of plaques at the time of endarterectomy (n=337), 65.0% of patients with ocular symptoms versus 70.1% with cerebral symptoms had evidence of ulceration (P=0.36). Although these indirect assessments of plaque vulnerability correlate well with our validated histological assessments, they are clearly not as sensitive and are more prone to observer bias.

We found that there were a greater number of plaque features believed to be associated with plaque vulnerability from patients with cerebral ischemic events compared with those with ocular events (adjusted ORs up to 1.7). Despite this, over a quarter of patients with recent ocular events did have ≥4 of these vulnerable plaque features. This subgroup may be at higher risk of future stroke without endarterectomy and could benefit most from early carotid endarterectomy. Future plaque imaging studies should focus on low-risk groups of patients, such as those with recent ocular events, to determine the prognostic value of imaging surrogates of these vulnerable plaque features.

Although we consider our results to be valid, our study did have certain limitations. First, the study was a collaboration between 2 large carotid plaque biobanks, and although patient characteristics and techniques used for processing and assessment of plaques were similar, some differences are noteworthy. The Oxford Plaque Study examined the entire specimen along the longitudinal axis with 3-mm intervals, whereas the Athero-Express Study examined the culprit lesion, adjacent segments being processed for protein extraction. However, both studies have shown previously that differences in sampling and sectioning technique do not have a major impact on categorization of plaque histology. Second, many plaques from the Oxford Plaque Study were collected in the 1980s and 1990s, after which the time from symptoms to surgery has been reduced and the use of antiplatelet agents and statins has increased. However, our findings in the 2 separate studies were similar despite these changes. Third, we cannot prove that the differences in plaque histology that we have identified between patients with ocular and cerebral events would be responsible for the well-documented differences in expected risk of stroke had the patients not had endarterectomy. However, it has been shown previously that the risk of ipsilateral ischemic stroke during the few years after endarterectomy is similar in the 2 patient groups, which supports the hypothesis that the difference in stroke risk in patients who do not have surgery is attributable to the plaque. The presence of intracranial atherosclerotic stenosis is known to be an independent risk factor for future ischemic stroke in medically treated patients with symptomatic carotid stenosis. The lack of intracranial arterial imaging on patients in this study prevents us from excluding this as a confounding factor. However, in NASCET, the prevalence of intracranial stenosis was similar in patients with ocular events only (32.5%) and those with transient cerebral events (30.8%). It is, therefore, unlikely that this factor has had any significance influence on our findings.

In conclusion, we have shown that carotid plaque composition differs between patients with recent ocular ischemic events versus cerebral ischemic events, which may help to explain the well-documented differences in stroke risk between these groups. However, the difference in rates of characteristics that are believed to be associated with plaque vulnerability between the groups was not substantial, and so other mechanisms might also be important, such as flow-dynamics and cellular ischemic tolerance in addition to plaque composition.

Acknowledgments

We are grateful to the staff of the Departments of Vascular Surgery and Histopathology at the John Radcliffe Hospital, Oxford, UK, and University Medical Center Utrecht and St Antonius Hospital, Nieuwegein, the Netherlands for the provision of plaques for this study.
Sources of Funding
D.P.J. Howard and the Oxford Plaque Study are supported by the National Institute for Health Research (NIHR) Biomedical Research Center Program (Oxford). The views expressed are those of the authors and not necessarily those of NIHR or UK Department of Health.

Disclosures
None.

References
Histological Features of Carotid Plaque in Patients With Ocular Ischemia Versus Cerebral Events

Dominic P.J. Howard, Guus W. van Lammeren, Jessica N. Redgrave, Frans L. Moll, Jean-Paul P.M. de Vries, Dominique P.V. de Kleijn, Gert Jan de Borst, Gerard Pasterkamp and Peter M. Rothwell

Stroke. 2013;44:734-739; originally published online January 29, 2013;
doi: 10.1161/STROKEAHA.112.678672

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/44/3/734

Data Supplement (unedited) at:
http://stroke.ahajournals.org/content/suppl/2013/01/29/STROKEAHA.112.678672.DC1

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/
**Web-table 1: Definitions for histological plaque features in Oxford Plaque Study and Athero-Express**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Staining Oxford</th>
<th>Staining AE</th>
<th>Definition used in Oxford Plaque Study</th>
<th>Definition used in Athero-Express</th>
<th>Overall Agreement</th>
<th>Similarities</th>
<th>Differences</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Overall plaque stability</strong></td>
<td>H&amp;E</td>
<td>H&amp;E</td>
<td>0 Stable predominantly fibrous plaque with thick, intact cap OR predominantly stable, some features of instability, eg, inflammation, but thick, intact cap</td>
<td>0 Stable/fibrous: small (&lt;10% of plaque area) or absent lipid core, low macrophage infiltration, and high smooth muscle cell and collagen content</td>
<td>Good</td>
<td>Assessment includes</td>
<td>- Cap rupture (no data in AE)</td>
</tr>
<tr>
<td></td>
<td>EVG</td>
<td>EVG</td>
<td>1 Unstable with intact thin cap, large lipid core, but no definite rupture or surface thrombus OR unstable with ruptured cap or thrombus present</td>
<td>1 Unstable/atheromatous: large lipid core (&gt;40% of plaque surface area) and high macrophage infiltration with low smooth muscle cell and collagen content</td>
<td>Good</td>
<td>- Lipid core</td>
<td>- Thrombus (not included in AE definition)</td>
</tr>
<tr>
<td></td>
<td>CD68</td>
<td>CD68</td>
<td></td>
<td></td>
<td>Good</td>
<td>- Fibrous composition</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PSR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Inflammation</td>
<td></td>
</tr>
<tr>
<td><strong>Fibrous</strong></td>
<td>H&amp;E</td>
<td>H&amp;E</td>
<td>0 Very little fibrous tissue or = 50% fibrous tissue</td>
<td>0 Atheromatous/fibroatheromatous</td>
<td>Good</td>
<td>Both based on 3-grade scale</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EVG</td>
<td>EVG</td>
<td>1 Predominantly fibrous plaque</td>
<td>1 Predominantly fibrous plaque, absent lipid core, or &lt;10% of plaque surface area</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lipid core</strong></td>
<td>H&amp;E</td>
<td>H&amp;E</td>
<td>0 None or small</td>
<td>0 no or smaller than 40% of total plaque surface area</td>
<td>Moderate / Good</td>
<td>Both based on 3-grade scale</td>
<td>Different cut off: Oxford &gt;25% AE &gt;40%</td>
</tr>
<tr>
<td></td>
<td>PSR</td>
<td></td>
<td>1 A large lipid core was considered to occupy ≥ 50% of the thickness of the plaque or ≥ 25% of the total section area.</td>
<td>1 &gt;40% of plaque surface area covered by lipid core</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Inflammatory plaque</strong></td>
<td>CD68</td>
<td>CD68</td>
<td>0 No staining OR +: occasional scattered cells or 1 group of &gt;50 cells</td>
<td>0 Absent or minor CD68 staining with negative or clusters with &lt;10 cells present</td>
<td>Good</td>
<td>Both based on 4-grade scale</td>
<td>Different cut off for number of positive cells and clusters</td>
</tr>
<tr>
<td><strong>(severity of CD68 staining)</strong></td>
<td>CD 3</td>
<td>CD 3</td>
<td>1 +++: Several groups (&gt;5) of &gt;50 cells or +++: Many groups (&gt;5) of &gt;50 cells or 1 group of &gt;500 cells</td>
<td>1 moderate or heavy staining, cell clusters with &gt;10 cells present or abundance of positive cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Presence of thrombus</strong></td>
<td>H&amp;E</td>
<td>H&amp;E</td>
<td>0 No luminal thrombus</td>
<td>0 No luminal thrombus</td>
<td>Good</td>
<td>- Comparable definitions</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Fibrin</td>
<td></td>
<td>1 Thrombus was recorded when there was an organized collection of fibrin and red blood cells in the lumen.</td>
<td>1 Thrombus formation at the luminal side of the plaque with positive staining for fibrin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Characteristic</td>
<td>Staining Oxford</td>
<td>Staining AE</td>
<td>Definition used in Oxford Plaque Study</td>
<td>Definition used in Athero-Express</td>
<td>Overall Agreement</td>
<td>Similarities</td>
<td>Differences</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>-----------------</td>
<td>-------------</td>
<td>---------------------------------------------------------------------------------------------------------</td>
<td>----------------------------------</td>
<td>-------------------</td>
<td>--------------</td>
<td>-------------------------------------------</td>
</tr>
<tr>
<td>Presence of intraplaque hemorrhage</td>
<td>H&amp;E</td>
<td>H&amp;E</td>
<td>0 None</td>
<td>0 Absent</td>
<td>Good</td>
<td>Loose</td>
<td>Additional fibrin staining in AE</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fibrin</td>
<td>1 Includes recent or old haemorrhage; is an area of erythrocytes causing disruption of plaque architecture (def: Bassiouney et al.)</td>
<td>1 Haemorrhage within the tissue of the plaque, including fresh and organized haemorrhage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcifications</td>
<td>H&amp;E</td>
<td>H&amp;E</td>
<td>0 None or small amounts when there was stippling only</td>
<td>0 no or minor staining along part of the luminal border of the plaque or a few scattered spots within the lesion</td>
<td>Good</td>
<td>Both</td>
<td>Based on 4-grade scale</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 considered to be present in large amounts when nodular deposits</td>
<td>1 moderate or heavy staining along the entire luminal border or evident parts within the lesion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Collagen</td>
<td>EVG</td>
<td>No data</td>
<td></td>
<td>0 no or minor staining along part of the luminal border of the plaque</td>
<td>None</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 moderate or heavy staining along the entire luminal border</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plaque rupture</td>
<td>EVG</td>
<td>0 Intact Cap</td>
<td></td>
<td>No data</td>
<td>None</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smooth muscle cells</td>
<td>A-actin</td>
<td>No data</td>
<td>0 no or minor -actin staining over the entire circumference with absent staining at parts of the circumference of the arterial wall</td>
<td>1 positive cells along the circumference of the luminal border, with locally at least few scattering cells</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>