Histological Characterization of Restenotic Carotid Plaques in Relation to Recurrence Interval and Clinical Presentation
A Cohort Study

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Backgrounds and Purpose—Restenosis is an important complication after carotid endarterectomy, but little is known about plaque composition in early versus late restenosis and which plaque characteristics are associated with symptomatic clinical presentation of restenotic lesions.

Methods—Endarterectomy specimens of 822 consecutive patients undergoing carotid endarterectomy (33 restenotic; 789 primary) were subjected to histological examination for the presence of macrophages, smooth muscle cells, collagen, calcifications, luminal thrombus, intraplaque bleeding and lipid core size.

Results—Early restenotic plaques showed marked accumulation of smooth muscle cells and fibrous tissue, whereas late restenotic plaques demonstrated increased macrophage infiltration, calcification and lipid core (P trend <0.05), resembling primary plaques. Patients with symptomatic restenosis had plaques with higher macrophage infiltration (P=0.01) and a larger lipid core (P=0.02) than asymptomatic patients, independent of recurrence interval.

Conclusions—Restenosis occurring >5 years after primary carotid endarterectomy resembles primary plaques. Symptomatic presentation of restenotic lesions is independently associated with an unstable plaque phenotype. (Stroke. 2008;39:1029-1032.)

Key Words: atherosclerotic plaque ■ carotid endarterectomy ■ cerebrovascular symptoms ■ histology ■ restenosis

Restenosis remains an important problem challenging the results of carotid endarterectomy (CEA). Restenosis occurs in about 10% of patients in the first year after CEA and in 20% renarrowing of the lumen occurs during longer follow-up.

Recent histopathologic studies revealed that primary carotid artery stenotic lesions that give rise to local thromboembolic events share a vulnerable plaque phenotype, characterized by a large lipid core and infiltration of inflammatory cells. However, it has not been studied if the restenotic plaque that is prone to cause a cerebrovascular event shares the same histological characteristics. Furthermore, it is unclear if early and late restenosis could be considered comparable or different processes. The objective of the present study was to investigate the characteristics of restenotic plaques in relation to recurrence interval and clinical presentation.

Methods
Consecutive patients who underwent CEA were included (April 2002 to February 2007) in the Athero-Express study. Written informed consent was obtained from all patients.

Definition of Restenosis
Restenosis was defined as a recurrent luminal narrowing of at least 50% (peak systolic velocity ≥125 cm/s; duplex ultrasound) in patients who had undergone prior carotid endarterectomy of the ipsilateral carotid artery. The indications for performing CEA in restenotic patients were cerebrovascular symptoms or a progressive asymptomatic lesion (≥70%; except near-occlusions). Based on recurrence interval we distinguished between early (<2 years), intermediate (2 to 5 years) and late restenosis (>5 years).

Plaque Characterization
Carotid endarterectomy was performed using selective shunting and selective patching. The excised plaques were directly transferred to the laboratory and processed and examined as described previously. Macrophages (CD68), smooth muscle cells (Alpha-Actin), collagen (Picro-Sirius) and calcifications (hematoxylin and eosin [HE]) were rated as no/minor or moderate/heavy. Luminal thrombus and intraplaque bleeding (HE, Elastin von Gieson) were rated as absent or present. Overall phenotype was based on lipid core size: fibrous (<10% of plaque area), fibro-atheromatous (10% to 40%), and atheromatous (>40%).

Statistical Analysis
Histological characteristics were compared between groups using the χ² test. For each plaque characteristic associated with symptomatic
presentation of restenosis, a logistic regression model was constructed to adjust for recurrence interval. The result was expressed as odds ratio (OR) with 95% CI. Probability values $<0.05$ were considered statistically significant.

**Results**

The patient group with recurrent stenosis encompassed more female (48% versus 30%; $P=0.01$) and asymptomatic patients (49% versus 22%; $P=0.001$) than the primary group. The prevalence of other risk factors and medication use did not differ. Histological examination revealed that restenotic plaques contained less calcification, more luminal thrombus and a smaller lipid core compared to primary plaques (Table). These characteristics were not related to patch use during the original CEA. Plaque composition was dependent on recur-

| Table. Comparison of Plaque Histology Between Primary and Restenotic Plaques |
|------------------|------------------|------------------|------------------|------------------|
|                   | Restenosis       | Primary          | $P$               | Recurrence Interv (trend) |
|                   | All  Early  Intermediate Late | All  Early  Intermediate Late | 0.03 0.13 | 0.02 0.05 |
| n                 | 33   6     11 16 789 | 33   6     11 16 789 |                   |                   |
| Macrophages†‡     | 66% 33% 73% 73% 60% | 66% 33% 73% 73% 60% | 0.83 0.13 |                   |
| Smooth muscle cells†‡ | 62% 100% 64% 50% 71% | 62% 100% 64% 50% 71% | 0.87 0.02* |                   |
| Collagen†‡        | 75% 100% 73% 66% 83% | 75% 100% 73% 66% 83% | 0.86 0.14 |                   |
| Calcification‖‡   | 12% 0% 0% 25% 62% | 12% 0% 0% 25% 62% | <0.001* 0.05* |                   |
| Luminal thrombus  | 65% 50% 82% 57% 26% | 65% 50% 82% 57% 26% | <0.001* 0.45 |                   |
| Intraplaque bleeding | 55% 50% 46% 64% 64% | 55% 50% 46% 64% 64% | 0.30 0.94 |                   |
| Overall phenotype | Fibrous 58% 83% 77% 35% 32% | Fibrous 58% 83% 77% 35% 32% | 0.008* 0.01* |                   |
|                   | Fibro-atheromatous 21% 17% 18% 25% 35% | Fibro-atheromatous 21% 17% 18% 25% 35% |                   |                   |
|                   | Atheromatous 21% 0% 9% 38% 33% | Atheromatous 21% 0% 9% 38% 33% |                   |                   |

*P<0.05; †percentages of plaques rated as “moderate” or “heavy” (eg, moderate or heavy infiltration of macrophages).

**Figure 1.** Plaque histology. Scalebars: 2 mm (A,B,C,E); 0.5 mm (D). A: Early restenotic plaque (Picro-Sirius staining). The plaque consists of fibrous tissue with massive accumulation of smooth muscle cells (see panel B). Note the patch at the top of the specimen. B: Smooth muscle cell accumulation in early restenotic plaque (Alpha-actin staining; brown). A large accumulation of smooth muscle cells is observed, which was present throughout the plaque. C: Late restenotic plaque (Picro-Sirius staining). Symptomatic restenosis 6 years after primary CEA. A large lipid core (left) is observed. Note the comparison with the primary plaque in panel E. D: Macrophage infiltration in the fibrous cap of a late restenotic plaque (CD68 staining; brown). E: Primary plaque (Picro-Sirius staining).
rence interval: early restenotic plaques showed a fibrous phenotype whereas late restenotic plaques appeared like primary lesions (Table; Figure 1).

We explored if differences in clinical presentation of restenotic plaques could be based on differences in plaque composition (Figure 2). Symptomatic plaques had higher infiltration of macrophages, a smaller smooth muscle cell component and larger lipid pools.

Symptomatic presentation was more frequently observed in the group with late restenosis compared to early restenosis, which matched well with the plaque characteristics associated with symptomatic recurrent disease. This could imply that the associations between symptomatic presentation of restenosis and plaque characteristics might be biased by the recurrence interval, which led us to adjust for recurrence interval. A paucity of smooth muscle cells was no longer associated in the adjusted analysis (OR=0.63 [0.12 to 3.3]). However, large lipid core showed a trend (OR=2.1 [0.67 to 6.24]) and macrophage infiltration was associated with symptomatic presentation of restenosis, independent of recurrence interval (OR=8.3 [1.2 to 59.4]; P=0.03).

Discussion

The present study defines histological characteristics of the vulnerable restenotic plaque. We show that in restenotic plaques, macrophage infiltration and large lipid core are associated with symptomatic presentation, comparable to primary plaques. These associations are independent of the recurrence interval.

The implication of these findings is that macrophage infiltration and lipid core size are the plaque characteristics that should be targeted with noninvasive plaque imaging to assess the risk of patients with recurrent carotid stenosis to become symptomatic. With present plaque imaging techniques, lipid core and macrophage infiltration can be detected quite reliably. Therefore, imaging studies to validate our observations are warranted.

The present study also clarifies that late restenosis (>5 years) is not clearly different from primary carotid artery stenosis, with marked macrophage infiltration, calcifications and lipid core. In contrast, early restenotic plaques are characterized by the presence of smooth muscle cells with absence of typical features of atherosclerotic plaques such as calcifications and a large lipid core. Because carotid plaque composition may influence peri-interventional complication rate and incidence of restenosis after the intervention, our results indicate that differentiating between early and late restenotic lesions could be important for clinical studies investigating treatment of restenosis.

In conclusion, the present study shows that restenosis occurring >5 years after primary carotid endarterectomy resembles primary plaques, and symptomatic presentation of early and late restenotic plaques is associated with high macrophage infiltration and large lipid core, independent of recurrence interval.
Disclosures
None.

References
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