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Carotid Atherosclerotic Plaque Characteristics Are Associated With Microembolization During Carotid Endarterectomy and Procedural Outcome

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Background and Purpose—During carotid endarterectomy (CEA), microemboli may occur, resulting in perioperative adverse cerebral events. The objective of the present study was to investigate the relation between atherosclerotic plaque characteristics and the occurrence of microemboli or adverse events during CEA.

Methods—Patients (n=200, 205 procedures) eligible for CEA were monitored by perioperative transcranial Doppler. The following phases were discriminated during CEA: dissection, shunting, release of the clamp, and wound closure. Each carotid plaque was stained for collagen, macrophages, smooth muscle cells, hematoxylin, and elastin. Semiquantitative analyses were performed on all stainings. Plaques were categorized into 3 groups based on overall appearance (fibrous, fibroatheromatous, or atheromatous).

Results—Fibrous plaques were associated with the occurrence of more microemboli during clamp release and wound closure compared with atheromatous plaques ($P=0.04$ and $P=0.02$, respectively). Transient ischemic attacks and minor stroke occurred in 5 of 205 (2.4%) and 6 of 205 (2.9%) patients, respectively. Adverse cerebral outcome was significantly related to the number of microembolic events during dissection ($P=0.003$) but not during shunting, clamp release, or wound closure. More cerebrovascular adverse events occurred in patients with atheromatous plaques (7/69) compared with patients with fibrous or fibroatheromatous plaques (4/138) ($P=0.04$).

Conclusions—Intraoperatively, a higher number of microemboli were associated with the presence of a fibrous but not an atheromatous plaque. However, atheromatous plaques were more prevalent in patients with subsequent immediate adverse events. In addition, specifically the number of microemboli detected during the dissection phase were related to immediate adverse events. **(*Stroke*. 2005;36:1735-1740.)**

Key Words: carotid artery plaque ■ carotid endarterectomy ■ embolism ■ microcirculation ■ stroke

Carotid artery stenosis is a common disease in Western society and related with the occurrence of transient ischemic attacks (TIA) and strokes.[1] Carotid endarterectomy (CEA) is a widely applied method to treat symptomatic and asymptomatic patients with severe carotid artery stenosis. However, 3% to 7% of the CEA procedures are complicated by disabling or nondisabling strokes.[1–3] Perioperative transcranial Doppler (TCD) registration of the middle cerebral artery provides online surveillance of both hemodynamic changes and passage of cerebral microemboli.[4–7] Therefore, TCD surveillance is widely applied during CEA.[4,8–10] Perioperative microembolization detected by TCD monitoring has been related with the occurrence of adverse neurological events.[11–13] Asymptomatic microemboli recorded by one-hour TCD registration in a group of nonoperated patients with symptomatic as well as asymptomatic carotid stenosis was found to be related to TIA and stroke during follow up.[14]

In cardiovascular disease, next to plaque size and luminal narrowing, plaque characteristics are also considered causally related to the development of cardiovascular events.[15–18] In general, the vulnerable unstable plaque consists of inflammatory cells, accumulated lipid, and a thin fibrous cap and is associated with plaque rupture, thrombosis, and subsequent myocardial infarction. The relation between carotid plaque characteristics, plaque embolization, and adverse clinical outcome is unknown.

In the current prospective study, we focused on the association between plaque phenotype and microembolic events registered by transcranial Doppler ultrasonography (TCD). In addition, plaque characteristics and the number of microembolic events were related to the occurrence of postoperative adverse ischemic cerebral events. In line with coronary atherothrombosis, we hypothesized that the inflammatory, atheromatous plaque is associated with an increased

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incidence of microemboli after carotid endarterectomy (CEA). Such a finding is important, because the development and increasing resolution of imaging techniques like MRI, ultrasonography, or multislice CT scanning may facilitate noninvasive detection of the vulnerable plaque.

Subjects and Methods

Patients

ATHERO-EXPRESS is an ongoing longitudinal study, with the objective of investigating the etiological value of plaque characteristics for long-term outcome. The design of the study has been described previously.[19] The ATHERO-EXPRESS study is currently being executed in 2 Dutch hospitals, UMC Utrecht and St. Antonius Hospital Nieuwegein. In brief, recruitment of patients started in April 2002 and will continue until at least 1000 patients have been enrolled. All consecutive patients who are newly referred to the vascular surgery departments of the participating centers for treatment of carotid artery stenosis are enrolled. Patients may have been symptomatic or asymptomatic. Operation is indicated when color Doppler–assisted duplex investigation reveals a diameter reduction of >70% on at least 1 side. In asymptomatic patients with stenosis >70%, the indication for surgical therapy depends on comorbidity and vertebrobasilar insufficiency. Two hundred patients with successful TCD monitoring throughout the entire operation entered this study.

Carotid Endarterectomy

All patients underwent operation while under general anesthesia. Patients were monitored by TCD and electroencephalographic (EEG) registration. A shunt was selectively used on the basis of EEG and TCD criteria, as described in earlier reports.[20,21] Before cross-clamping, an intravenous bolus of heparin (5000 IU) was administered. One hundred ninety-seven patients (99.5%) used antiplatelet agents (aspirin, Plavix, persantine, or a combination of these) or coumarin medication preoperatively. All endarterectomies were open with careful dissection of the bifurcation into the internal and external carotid arteries. When the vascular surgeon indicated patch closure, venous patches were preferred. A Dacron patch was used only when there was insufficient venous material.

Atherosclerotic Tissue Dissection and Processing

After dissection, the atherosclerotic plaque segment was transported without delay to the laboratory. The atherosclerotic plaque was cut in 0.5-cm segments. The culprit lesion was designated as segment 0 and the adjacent segments as −1 and +1. The segments −1, +1, and all subsequently numbered segments (−2, +2, −3, etc) were immediately frozen in liquid nitrogen and stored at −80°C. Segment 0 was fixed in 4% formaldehyde and embedded in paraffin. From each segment, 15 sections (5 μm) were cut for histological (immuno) staining. The following stainings were performed to characterize the plaque: picro-Sirius red (collagen and fat, determined by polarized light), CD68 (macrophages), α-actin (smooth muscle cells), hematoxylin (thrombus and calcifications), and elastin von Gieson's (internal elastic lamina).

Plaque Phenotyping

Two independent observers microscopically scored all stainings semi-quantitatively as described earlier.[17] A plaque was considered unstable when it contained high numbers of macrophages and a large atheroma and when it lacked collagen and smooth muscle cells. The more fibrous stable lesions typically lacked inflammatory cells and fat and reveal strong staining for collagen and smooth muscle cells. Plaques were categorized as no/minor staining or moderate/heavy staining for the stains listed as follows: (A) Collagen staining by polarized light microscopy: (1) no or minor staining along part of the luminal border; (2) moderate or heavy staining along the entire luminal border; (B) CD68-positive cells: (1) absent or minor staining with negative or few scattered cells; (2) moderate or heavy staining, clusters of cells with >10 cells present; (C) α-Actin–positive cells: (1) no or minor staining over the entire circumference with absent staining at parts of the circumference of the arterial wall; (2) positive cells along the circumference of the luminal border, with locally at least minor staining with a few scattered cells; and (D) Hematoxylin: (1) no signs of earlier intraplaque thrombus formation; (2) signs of earlier thrombus formation (fibrin deposition).

The percentage of atheroma within the total area of plaque was visually estimated from the picro-Sirius red stain with polarized light and hematoxylin stains. Two groups were considered on the basis of the percentage of atheroma in the plaque: >40% and <40%.[20] Plaques were also categorized into 3 groups based on their overall appearance (fibrous, fibroatheromatous, or atheromatous).

TABLE 1. Baseline Patient Characteristics

No. of patients	200
No. of operations	205
Sex, male/female	136 (68.7%)/62 (31.3%)
Age, median (range), y	68 (41–86)
Smoking, yes/no	49 (29%)/121 (71%)
Diabetes, yes/no	38 (23%)/130 (87%)
Dyslipidemia, yes/no	101 (60%)/60 (40%)
Symptoms, yes/no	148 (72%)/57 (28%)
Side operated, left/right	109 (53%)/96 (47%)
Shunt used	53 (26%)

TCD Monitoring

The methods of TCD monitoring have been reported previously.[21,22] In brief, blood flow velocities were measured in the middle cerebral artery. The probe was affixed to the lateral temporal region. Doppler signals were recorded, and high-intensity transient signals indicating microemboli were identified. In the present study, the Doppler spectra were observed in the operating room by an experienced

TABLE 2. TCD-Recorded Microemboli During Different Phases of Operation

Dissection phase	
No. of patients with embolic event	27% (55/205)
Mean (SEM)	1.43 (0.39)
Range	0–60
Total	294
Shunt phase	
No. of patients with embolic event	53% (27/51)
Mean (SEM)	5.16 (1.20)
Range	0–48
Total	263
Clamp release phase	
No. of patients with embolic event	54% (111/205)
Mean (SEM)	7.27 (1.32)
Range	0–180
Total	1482
Total No. of patients with shower	24 (91 showers)
Wound closure phase	
No. of patients with embolic event	32% (65/205)
Mean (SEM)	1.4 (0.26)
Range	0–28
Total	294

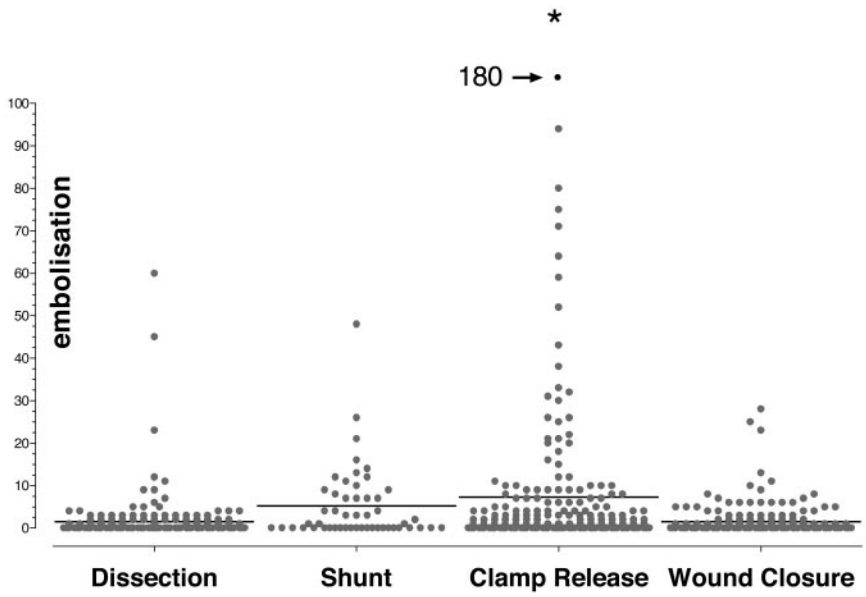

Figure 1. Embolization distribution for the different phases of operation: dissection, shunting, clamp release, and wound closure. Black bars indicate the mean. *$P<0.05$

sonographer. All microembolic events were counted during 4 different phases during operation: (1) dissection (all microembolic events during skin incision until cross-clamping), (2) shunting (when a shunt was used, microemboli that occurred during introduction until removal of the shunt), (3) clamp release (the first 10 seconds after restoration of flow through the carotid arteries), and (4) wound closure (after the first 10 second of flow restoration until end of operation). Microemboli that occurred during release of the clamp and that could not be counted separately during 1 heartbeat were designated shower microemboli. A shower of microemboli was assigned an arbitrary number of 10 microemboli, which is the maximum number of microemboli that may be discriminated during 1 heartbeat.

Outcome

Patients' hospital records were used to obtain information about clinical outcome. A neurologist was routinely consulted for all patients preoperatively and at the third day after operation. New neurological symptoms or worsening of existing symptoms persisting for >24 hours were regarded as stroke. Stroke was classified according to the modified Rankin Scale.[23] New neurological symptoms persisting <24 hours were regarded as a transient ischemic attack (TIA). Adverse ischemic cerebral events were counted when they were diagnosed postoperatively up to 2 weeks after operation.

Data Analysis

Data are presented as mean±SEM. We used nonparametric tests for continuous variables (Mann–Whitney test, Wilcoxon signed-rank test, and Friedman's test), and for categorical variables, we used χ^2 and Fisher's exact test. Probability values <0.05 were considered statistically significant.

Results

Table 1 shows baseline patient characteristics. Table 2 shows the number of TCD-registered microemboli for the different operative phases. Significantly more microemboli were recorded during clamp release compared with the other phases of operation (Figure 1).

The relation between the presence of microemboli and overall plaque characteristics for the different operative phases is shown in Table 3 and Figure 2. During clamp release as well as wound closure, the presence of microemboli was found to be associated with fibrous plaque ($P=0.04$ and $P=0.02$, Table 3). The relation between plaque characteristics and microemboli are presented in Table 4. No or minor calcified plaques were related to a greater number of microemboli during dissection. In accordance with overall plaque phenotype, the presence of <40% atheroma in the plaque was also associated with significantly more microem-

TABLE 3. Overall Plaque Phenotype

Fibrous	
Percentage of plaques	30.4% (61/201)
Embolic events	
Dissection	0.90 (0.27)
Shunting	6.27 (2.10)
Clamp release	7.00 (1.85)*
Wound closure	2.34 (0.65)†
Patients with adverse neurological outcome	1/61 (1.6%)‡
Fibroatheromatous	
Percentage of plaques	35.3% (71/201)
Embolic events	
Dissection	1.13 (0.40)
Shunting	5.56 (2.98)
Clamp release	10.38 (3.19)*
Wound closure	1.30 (0.30)†
Patients with adverse neurological outcome	3/71 (4.3%)‡
Atheromatous	
Percentage of plaques	34.3% (69/201)
Embolic events	
Dissection	2.30 (1.07)
Shunting	4.00 (1.23)
Clamp release	4.42 (1.39)*
Wound closure	0.51 (0.140)†
Patients with adverse neurological outcome	7/69 (10.1%)‡

Data are mean and (SEM).

*$P=0.04$; †$P=0.02$; ‡$P=0.09$ (when data for fibrous and fibroatheromatous are pooled, $P=0.04$).

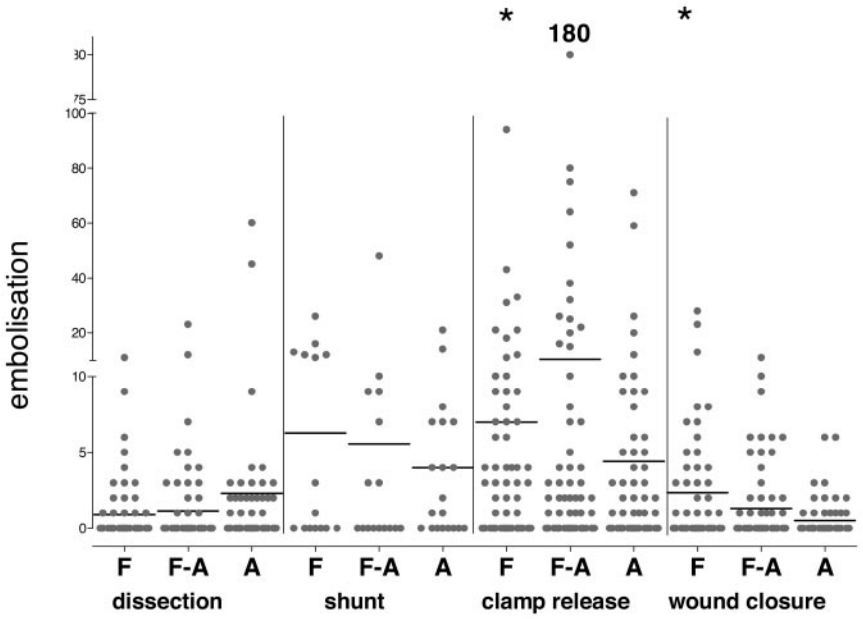

Figure 2. Embolization during dissection, shunting, clamp release, and wound closure in relation to plaque phenotype. During clamp release and wound closure, fibrous plaques had significantly more microembolization then atheromatous plaques ($P=0.04$ and $P=0.02$). Black bars indicate mean values. Note the *y*-axis scaling.

boli during wound closure ($P=0.02$). The number of microemboli was not associated with the presence of macrophages (Tables 3 and 4).

Adverse ischemic cerebral events occurred in 2.4% (TIA) and 2.9% (minor stroke) of patients and were related to significantly more microemboli during dissection (0.92 vs 6.91, $P=0.003$) but not during clamp release or wound closure. More adverse events occurred in patients with atheromatous plaque (7/69 plaques) compared with patients with fibrous or fibroatheromatous plaque (4/138, $P=0.04$). There were no significant differences in the incidence of microemboli among surgeons who performed the operation (not shown).

Discussion

Carotid artery stenosis is a common presentation of atherosclerotic disease.[1] Nine percent to 12% of patients with known atherosclerotic disease have a high-grade carotid artery stenosis.[24] CEA is a widely accepted method to treat patients with carotid artery stenosis. Reduction of perioperative morbidity and mortality could improve long-term outcome. It has been demonstrated that high microembolic rates during CEA are related to adverse neurological outcome.[11–13] In cardiovascular disease, the vulnerable inflammatory atheromatous plaque is considered responsible for thrombotic events and subsequent myocardial infarction.[15,16,18] We hypothesized that a vulnerable plaque phenotype could also be associated with the occurrence of microemboli during CEA.

Surprisingly, in this study, we found a relation between the presence of fibrous plaque and the incidence of microemboli after unclamping and wound closure. In agreement with this finding, plaques containing <40% fat were also related to a greater number of microemboli during wound closure. There was no relation between emboli and fibrous or fibroatheromatous plaques during dissection and shunting. In line with our hypothesis, adverse neurological outcomes were related to an increase of microembolic events during dissection of the artery. Atheromatous plaque phenotype was more prevalent in patients with an adverse event.

Plaque Characteristics

In coronary artery disease, a strong relation has been described between plaque characteristics and plaque thrombosis; deposition of free cholesterol, macrophage infiltration, enlargement of the necrotic core, and a thin fibrous cap are features that are related to the instable or vulnerable plaque.[15–18] Surprisingly, we observed an association between microembolic events and stable fibrous plaques instead of inflammatory lipid-rich plaques during unclamping and wound closure. We must take into consideration that the origin of microemboli likely alters during operation. Before arteriotomy, emboli are likely attributable to plaque debris, whereas during and after arteriotomy, air emboli are likely to occur. In addition, removal of a (fibrous) plaque may expose collagen to flowing blood, with the subsequent formation of fresh thrombi as a result. Similar to atheromatous tissue, collagen is also known for its thrombotic capacity.[25] Atherosclerotic plaques with less calcification were associated with an increased number of microemboli during the dissection phase. This is in line with previous observations that showed that less calcified plaques have been associated with symptomatic carotid artery stenosis.[26]

Outcome

In this study, we showed a significant relation between microembolic events during dissection and the occurrence of adverse neurological events. The relation between microembolic events and adverse neurological outcome has been described by other authors.[11,12] In our study, 11 adverse events (TIA and minor stroke) were recorded, a percentage that is comparable to that in previous studies.[1–3] Although the total microembolus rate was not associated with an atheromatous plaque phenotype, the presence of atheromatous plaques was related to adverse neurological

TABLE 4. Plaque Characteristics in Relation to Embolic Events

	No/Minor Staining	Moderate/Heavy Staining	*P* Value
Dissection			
Percentage atheroma*	1.46 (0.52)	1.44 (0.67)	0.30
Calcification staining	2.30 (0.77)	0.57 (0.15)	0.02
Collagen staining	1.67 (0.94)	1.39 (0.45)	0.60
Macrophage staining	1.12 (0.29)	1.73 (0.78)	0.81
SMC staining	2.43 (1.12)	0.96 (0.18)	0.59
Thrombus†	0.53 (0.14)	1.85 (0.59)	0.25
Shunt phase			
Percentage atheroma*	5.67 (1.71)	4.22 (1.36)	0.83
Calcification staining	3.81 (1.18)	6.56 (2.11)	0.42
Collagen staining	2.08 (0.790	6.10 (1.53)	0.3
Macrophage staining	3.59 (0.90)	7.23 (2.40)	0.34
SMC staining	4.00 (1.25)	5.90 (1.81)	0.86
Thrombus†	1.78 (1.32)	5.88 (1.41)	0.13
Clamp release			
Percentage atheroma*	8.74 (1.94)	4.74 (1.43)	0.17
Calcification staining	8.42 (2.22)	6.25 (1.52)	0.71
Collagen staining	5.69 (2.29)	7.96 (1.67)	0.5
Macrophage staining	8.10 (1.77)	6.79 (2.14)	0.3
SMC staining	7.23 (2.19)	7.58 (1.77)	0.27
Thrombus†	8.97 (3.37)	6.69 (1.34)	0.97
Wound closure			
Percentage atheroma*	1.81 (0.35)	0.50 (0.15)	0.02
Calcification staining	1.55 (0.37)	1.16 (0.29)	0.91
Collagen staining	0.73 (0.20)	1.59 (0.31)	0.56
Macrophage staining	1.63 (0.42)	1.03 (0.22)	0.32
SMC staining	0.84 (0.27)	1.67 (0.34)	0.1
Thrombus†	1.12 (0.31)	1.47 (0.32)	0.58

Data are mean and (SEM). SMC indicates smooth muscle cell.
*Percentage <40% fat or >40% fat.
†Thrombus present (no or yes).

outcome. The latter would be in agreement with the idea that this plaque phenotype is related to thromboembolic events and hence, to adverse outcome. In the dissection phase, no association was found between fibrous plaques and microemboli. In contrast, atheromatous plaques tended to be associated with more embolic events in the dissection phase. This suggests that microembolic events during the dissection phase, together with the presence of an atheromatous plaque, strongly increase the risk for the development of adverse neurological events during and immediately after CEA.

Limitations

Our study is limited by the number of adverse events and by the interpretations of the TCD-registered microembolic event. Because TCD is based on ultrasound, it is not possible to discriminate between different kinds of embolic events (particles of the plaque, thromboemboli, and air emboli) during the operation. We assume that microemboli during dissection are mainly plaque particles and during wound closure are mainly thromboembolic, whereas during clamp release, embolic events are likely air.[12] For this reason, we divided embolic events during operation in the aforementioned categories. The duration of TCD registration is another potential limitation, because postoperative TCD monitoring was not performed.

We assumed that after CEA, plaque remnants at the edges reflect the same characteristics as the culprit lesion. This assumption is not proven, because plaque phenotype can be quite heterogeneous and therefore merits careful consideration. However, the strongest predictive plaque marker for adverse outcomes was observed in the dissection phase when plaques were still in situ.

Conclusions

Fibrous plaques are associated with an increase in microembolization during clamp release and wound closure but not with immediate adverse outcome. On the other hand, the presence of an atheromatous plaque, together with an embolic event during dissection, was related to the occurrence of TIA or minor stroke. The presence of local inflammatory cells was not associated with embolization or adverse outcome. Imaging modalities capable of visualizing the atheromatous lesion and perioperative embolization may help to predict the development of adverse neurological events after CEA.

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