Cerebral Protection During Carotid Artery Stenting
Collection and Histopathologic Analysis of Embolized Debris

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Background and Purpose—Histopathologic analysis was performed to better understand quantity, particle size, and composition of embolized debris collected in protection filters during carotid artery stent implantation.

Methods—Elective carotid stent implantation with the use of a distal filter protection was attempted in 38 consecutive lesions (36 patients) of the internal carotid artery presenting >70% diameter stenosis (mean, 82.1±11.1%). Mean age of the patients was 70.7±7.7 years; 75% were men, and 50% of patients had previous neurological symptoms.

Results—In 37 lesions (97.4%) it was possible to position the filter device, and in all lesions a stent was successfully implanted. The only complication occurring in the hospital and during the 30-day follow-up was 1 death due to acute myocardial infarction. Neurological sequelae did not occur. Presence of debris was detected in 83.7% of filters. The mean surface area of the polyurethane membrane filter covered with material was 53.2±19.8%. Particle size ranged from 1.08 to 5043.5 μm (mean, 289.5±512 μm) in the major axis and 0.7 to 1175.3 μm (mean, 119.7±186.7 μm) in the minor axis. Collected debris consisted predominantly of thrombotic material, foam cells, and cholesterol clefts.

Conclusions—By the use of distal protection filters during carotid artery stenting, it was possible to collect particulate debris potentially leading to distal vessel occlusion in a high percentage of cases. Qualitative analysis of embolized material showed debris dislocated during the percutaneous intervention from atheromatous plaques. (Stroke. 2002;33:456-461.)

Key Words: angioplasty ■ carotid arteries ■ embolism ■ pathology ■ stents

Stent placement for extracranial carotid artery disease has emerged as a potential alternative to carotid endarterectomy, the current gold standard treatment for carotid artery stenosis.1–3 However, compared with the surgical approach, percutaneous carotid stenting is accomplished with an increased incidence of microemboli, as shown by transcranial Doppler monitoring.4 These emboli are associated with a higher neurological complication rate5 and are also recognized as a potential cause of periprocedural stroke during endarterectomy.6–9

Only a few studies give a detailed morphological evaluation of the material retrieved during percutaneous intravascular procedures.10–15 From these studies it appears that most of the embolic dissemination occurs during iatrogenic manipulation of the atheromatous plaque.

Protection devices have the potential to reduce the incidence of intracranial debris embolization and render percutaneous carotid artery revascularization safer.12–19 In light of novel observations made by Reimers et al18 suggesting the safety and feasibility of filter devices for carotid artery stenting, the present prospective study was designed to analyze histopathologically debris collected during intravascular percutaneous carotid artery stent implantation with the use of a recently available protection device.

Subjects and Methods

Patient and Lesion Characteristics

Between September 2000 and February 2001, elective carotid artery stent implantation with the use of a distal protection filter was attempted in 38 lesions in 36 consecutive patients. These were the most recently treated patients and represent 38% of all lesions treated in our center to date. Eighteen patients (50%) had previous symptoms of transient ischemic attack and/or stroke. All 37 lesions produced a >70% diameter stenosis (mean, 82.1±11.1%) of the internal carotid artery. Thirty-seven lesions were de novo lesions, and 1 was restenosis occurring after endarterectomy. The clinical and angiographic characteristics are listed in Table 1.

Angiographic Evaluation

Baseline and postprocedural quantitative angiography was performed online with the use of the automated analysis system coordinated with the angiographic equipment (Integra 3000, Phil...
ips, Medical Instruments). The angiographic diameter stenosis was measured according to the North American Symptomatic Carotid Endarterectomy Trial (NASCET) criteria with the distal, nontapering portion of the internal carotid artery used as the reference segment. Qualitative angiographic evaluation was performed offline by 2 independent operators according to the criteria described by Mathur et al.

Drug Regimen

All patients were taking aspirin (100 to 325 mg), Ticlopidine (500 mg) was started at least 48 hours before the procedure. Heparin 70 to 100 U/kg was given intra-arterially to achieve an activated clotting time of >250 seconds. In case of inadequate anticoagulation, additional boluses of 2000 U of heparin were given until the target value was achieved. Atropine 1 mg was administered only when required to treat bradycardia during balloon inflation. Arterial blood pressure was monitored during the procedure and, if needed, was modulated with nitrates or dopamine. After the procedure, patients received aspirin indefinitely and ticlopidine for 1 month. Glycoprotein IIb/IIIa inhibitors were not used.

Carotid Stenting Procedure

Percutaneous access was gained through the femoral or brachial (1 lesion) artery. Long sheaths (6F or 7F) or coronary guiding catheters (8F) were advanced into the common carotid artery on long 0.035-inch support wires previously placed in the external carotid artery with the use of 4F or 5F diagnostic catheters. Angiography of the carotid artery and the intracranial circulation was performed in angulated views (Figure 1). Stenoses were crossed with the filter protection device. After filter opening, predilatation with 3.5- to 4.0-mm-diameter coronary angioplasty balloons was performed in 60.5% of the lesions at medium-low pressure (6 to 10 atm) until complete expansion of the balloon was seen. Appropriately sized self-expandable stents (Carotid Wallstent, Boston Scientific–Schneider; Smart 0.018 inch, J&J Cordis) were implanted, covering the carotid bifurcation in all cases. All stents were postdilated with the use of 5.5- to 7.0-mm (mean, 6.06±0.62 mm) balloons at medium-high pressures (8 to 12 atm; mean, 11.0±0.1). The arterial sheaths were removed on the same day. After the procedure, patients were transferred to the cardiology ward with continuous ECG monitoring for the following 12 hours, and noninvasive blood pressure measurements were taken every 2 hours for at least 12 hours.

Filter Device

The first-generation filter device (Angioguard, J&J Cordis) consists of an olive-shaped nitinol skeleton covered at its distal portion by a polyurethane membrane with pores of 100 μm in diameter. The filters used had diameters of 6, 7, and, in 1 case, 8 mm. The device is connected to a proximal, floppy wire tip and a distal 0.014-inch wire shaft 300 cm in length used as guidewire for the interventional procedure.

The closed filter, contained in a delivery sheath (diameter of 0.061 to 0.065 inch) with a maximum profile of 4F to 4.5F, was advanced through the lesion. The filter was opened in the internal carotid artery distal to the lesion by removal of the delivery sheath. A filter 0.5 to 1.0 mm larger than the visually estimated distal vessel diameter was chosen. This was to ensure complete apposition of the filter to the vessel wall. At the end of the procedure, a retrieval sheath was advanced, and the filter was closed and removed from the artery. Visual inspection of the filter was performed by the interventional cardiologist at the end of each procedure to evaluate the macroscopic presence of material.

Neurological Evaluation

A board-certified neurologist performed a complete neurological examination, including the National Institutes of Health Stroke Scale, before and after stent implantation.

Follow-Up

Clinical follow-up was performed 30 days after the procedure in all patients.

Histopathologic Evaluation

After removal, the filters were immediately fixed in 10% neutral buffered formalin. Photographs of the device in its integrity and after removal of the metallic wire were taken under a stereo microscope. These images were used for the computerized morphometric analysis with Image-Pro Plus software (Media Cybernetics). The total area of the filter membrane and the area covered by particulate material were quantified. The percentage of membrane occupied by debris was expressed as percentage of covered surface area. According to the extension of the covered area, patients were divided into 4 groups: group 1 had a covered area of ≤25%, group 2 >25% and ≤50%, group 3 >50% and ≤75%, and group 4 >75%. If particles were entrapped in the scaffolding metallic wires, they were carefully removed and separately photographed. Particulate floating in the formalin was filtered and processed for histological characterization. Particles identifiable as such were outlined by an experienced pathologist. The particles were subsequently counted and measured by the computer software. The 2 longest perpendicular diameters of

<table>
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<th>TABLE 1. Clinical and Angiographic Characteristics</th>
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<tr>
<td>Patients (n=36)/ Lesions (n=38)</td>
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<tr>
<td>Mean age, y 70.7±7.7</td>
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<tr>
<td>Male sex 27 (75%)</td>
</tr>
<tr>
<td>De novo lesions 37 (97.4%)</td>
</tr>
<tr>
<td>Previous endarterectomy 1 (2.6%)</td>
</tr>
<tr>
<td>Previous stroke/TIA 18 (50%)</td>
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</table>
| Angiographic evaluation  
| Mean % diameter stenosis 82.1±11.1            |
| Irregular lesion 28 (73.7%)                    |
| Ulcerated lesion 15 (39.5%)                    |
| Lesion calcification 15 (39.5%)                |
| Bilateral significant (>70%) carotid disease 12 (31.6%) |

TIA indicates transient ischemic attack.
the largest particles were recorded (Figure 2). All measurements were made at least twice.

For histology and tissue characterization, the material was removed from the filter, dehydrated, and embedded in paraffin, and the sections (5 μm thick) were stained with hematoxylin-eosin and Heidenhain trichrome.

Scanning electron microscopy was performed on the internal surface of the polyurethane membrane after it had been flattened. After removal from the 10% buffered formalin, the material was washed first in normal saline solution and then in distilled water. Subsequently, it was dehydrated in crescent series of alcohol, with a final incubation in amyl acetate, and processed for the CO2 critical dry point and gold sputtering. The specimens were observed under a scanning electron microscope (Philips XL 30).

Statistical Analysis
The data were computerized and analyzed with the use of the GraphPad Prism Software package. Mean, SD, and lower and upper 95% CIs were calculated for all parameters. Results are expressed as mean ± SD. \( P<0.05 \) was considered significant.

Results
Procedural and Follow-Up Results
In all patients it was possible to position a stent at the lesion site (Figure 1). In 1 case (2.6%) the filter could not be positioned, and an occlusive balloon protection was adopted (Guardwire, Percusurge Inc). In 5 cases (13.5%) the filter device crossed the lesion only after predilatation with the use of 2.0-mm-diameter coronary balloons. Distal vessel spasm, which resolved after intra-arterial nitrate administration, was observed during 3 procedures (8.1%). In 6 cases (16.2%) slow flow and in 2 cases (5.4%) no flow in the internal carotid artery occurred. The flow impairment resolved completely after filter removal in all 8 cases. Mean postprocedural diameter stenosis was 12.8 ± 9.2%. Procedural data and results are shown in Table 2.

Careful neurological examination, including the National Institutes of Health Stroke Scale, performed after the procedure, at discharge, and during the follow-up visit at 30 days did not reveal the occurrence of any neurological complications. Two patients required prolonged intravenous dopamine infusion for persistent hypotension. The 30-day mortality rate was 2.8% (1 patient). This patient with unstable coronary disease suffered massive anterior myocardial infarction 4 days after the procedure and died 3 days later.

Histopathological Results
Microscopic evaluation revealed that particles could be detected in 31 of 37 filters (83.7%) and that they were adherent mostly to the filter device (Figure 2). The mean number of particles in these filters was 33.7 ± 5.6 (range, 24 to 46). In 13 of 37 filters (35%), additional material was entangled in the scaffolding metallic wire. Only a small number of immersed particles were retrieved from the fixative. The average covered area on the plastic filter was 53.2 ± 19.8%. Mean particle size ranged from 1.08 to 5043.5 μm (mean, 289.5 ± 512 μm) in the major axis and from 0.7 to 1175.3 μm (mean, 119.7 ± 186.7) in the minor axis (Figure 2b). The distribution of the size of the particles is shown in Figure 3, and results of the histopathological analysis are shown in Table 3. Scanning electron microscopy was performed, and mean particulate size...
size of $311.6 \pm 251.5 \, \mu m$ in the major axis and $257.76 \pm 247.9 \, \mu m$ in the minor axis was observed (Figure 4). In all 8 filters of patients in which slow flow or no flow was observed, embolic material was detected, with an average covered area of $53.2 \pm 14.8\%$. Moreover, no relations could be detected between particle size and average covered area occupied by the material on the filters ($P = NS$).

Qualitative analysis with light microscopy revealed that the particles consisted primarily of soft acellular and amorphous material characterized by lipid-rich macrophages and cholesterol clefts. To a minor degree, calcium particulates and platelets entrapped in fibrin strands were found (Figure 5).

**Discussion**

Filter protection during carotid artery stenting has been recently reported by our group to be feasible and safe in a small cohort of patients with a low incidence of neurological complications.\(^{18}\)

The present study shows that embolic particulates that are commonly released during carotid artery stent implantation\(^3,4\) can be captured by distal filter protection devices. This is the clinical confirmation of the experience with filter protection in the ex vivo model reported by Ohki et al.\(^{13}\) Debris was captured in 83.7% of procedures compared with 81%\(^{12}\) and 100%\(^{14,15}\) in previously published studies in which occlusive balloons were used for protection. The size of the particles detected in the present study ($289.5 \pm 512 \, \mu m$ in diameter in the major axis; range, 1.08 to 5043.5 $\mu m$) was greater compared with the ex vivo filter evaluation ($226 \pm 130 \, \mu m$)\(^{13}\) and 1 of the distal occlusive balloon studies (54.4 mm crystal and 110.67 mm lipoid mass)\(^5\) and smaller compared with another distal balloon protection study (800 $\mu m$).\(^{14}\) In our experience, as well as in all previous observations, particles $>300 \, \mu m$ were found in all filters with debris,\(^{12-15,18}\) The microcirculation encompasses vessels with diameter $<300 \, \mu m.$\(^{22}\) The arterial network arborizes from the aorta to arteries up to 400 to 300 $\mu m$, small arteries with diameter between 300 and 100 $\mu m$, arterioles with diameter between 100 and 12 $\mu m$, and capillaries with diameter of approximately 12 $\mu m$. In the present study 100% of filters with materials presented particles $>300 \, \mu m$, and 52% of filters presented particles

<table>
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<tr>
<th>TABLE 3. Pathological Data</th>
<th>Filters (n=37)</th>
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<tr>
<td>Macroscopic presence of debris</td>
<td>27 (72.9%)</td>
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<tr>
<td>Presence of particulate material at microscopic evaluation</td>
<td>31 (83.7%)</td>
</tr>
<tr>
<td>Mean % covered area on filter</td>
<td>53.2±19.8</td>
</tr>
<tr>
<td>Mean % covered area on filter per group</td>
<td></td>
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<tr>
<td>Group 1: covered area $\leq 25%$ (3.4±6.1)</td>
<td>8* (21.6%)</td>
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<tr>
<td>Group 2: covered area $&gt;25$–$\leq 50%$ (37.8±7.9)</td>
<td>10 (27.0%)</td>
</tr>
<tr>
<td>Group 3: covered area $&gt;50$–$\leq 75%$ (63.6±4.5)</td>
<td>15 (40.5%)</td>
</tr>
<tr>
<td>Group 4: covered area $&gt;75$–$\leq 75%$ (79.2±3.9)</td>
<td>4 (10.8%)</td>
</tr>
<tr>
<td>Mean particle size, $\mu m$</td>
<td></td>
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<tr>
<td>Major axis</td>
<td>289.5±512</td>
</tr>
<tr>
<td>Minor axis</td>
<td>119.7±186.7</td>
</tr>
<tr>
<td>Mean no. of particles per filter</td>
<td>33.7±5.6</td>
</tr>
<tr>
<td>Filters with particles $&gt;300 , \mu m$, major axis</td>
<td>31/31 (100%)</td>
</tr>
<tr>
<td>Filters with particles $&gt;1000 , \mu m$, major axis</td>
<td>16/31 (51.6%)</td>
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*Including 6 filters without particulates.
It cannot be excluded that some of the retrieved thrombotic material could have been locally produced inside the filter, explaining the relatively high incidence (21.1%) of flow impairment and the high filter surface coverage of 50%, which was not correlated to the size of particles. As for the amorphous material, we cannot see any source other than the amorphous material dislocated from the atheromatous core of the plaque. All cases of transient flow impairment due to pore occlusion occurred immediately after postdilatation of the stent. Therefore, it appears that most emboli are released during this part of the procedure. Possible emboli release may occur during the passage of the closed filter device through the stenosis, and in the absence of intracranial Doppler we cannot exclude it. Protection systems such as low-profile distal occlusion balloons or proximal occlusion systems, which do not require any lesion crossing without protection, have the potential to reduce embolization during this procedural step.

It is, however, assumable that not all captured particles would have had acute clinical sequelae. The significance of clinically silent embolization during carotid artery interventions has not yet been established, and possibly microvascular obstructions are difficult to recognize. According to recent observations, emboli can potentially trigger platelet aggregation and may amplify microvascular obstructions. A shower of small particulate matter could cause microvascular obstruction. Furthermore, particles of vasoactive substances contained within plaque or thrombus could cause intense, prolonged vasospasm and subsequent cerebral infarction.

The histopathologic analysis showed that retrieved material consists of dislocated debris featuring atheroembolism as a consequence of atheromatous plaque squeezing and rupture occurring during carotid stenting. This result confirms the data of previously reported studies evaluating different emboli protection devices during percutaneous intravascular interventions. Our study confirms that this method of cerebral protection is feasible and safe and that it limits intracranial debris embolization. Therefore, it strongly supports the use of protection devices during percutaneous carotid stenting. However, we could not exclude the development of a stroke even in the setting of distal protection. In a recently published article, Roubin et al reported the important reduction of peri procedural complication rate over 5 years of experience from 9.3% in the first year to 4.3% in the fifth year. This was probably due to refinement of stenting techniques and newly developed equipment. We cannot rule out, as suggested by Qureshi et al, that the cardiac death
occurring in our study within the 30-day window may be in some way related to the hemodynamic instability after carotid stenting. To achieve the target of ≤3% periprocedural complication rate recommended by the American Heart Association/Society of Vascular Surgery guidelines for the treatment of asymptomatic patients, cerebral protection may well be essential.

Limitations of the Study
Because of the absence of intracranial Doppler monitoring during the stent procedure, this study does not exclude the presence of emboli not captured by the filter. In a recent ex vivo study, the filter device captured 88% of emblzed particles. Moreover, the small study size does not allow conclusions regarding the clinical efficacy of protection filters. Larger evaluations are warranted.

Conclusions
The present study demonstrates that protection filters collect debris of significant quantity and size during carotid artery stent implantation. Histopathological analysis showed that the collected material is dislocated from the atherosclerotic plaque during the intravascular procedure. Protection devices have the potential to further reduce neurological complications during the treatment of carotid artery stenosis.

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