Histopathology of Embolic Debris Captured During Transcatheter Aortic Valve Replacement

Nicolas M. Van Mieghem, MD; Marguerite E.I. Schipper, MD; Elena Ladich, MD; Elham Faqiri, MSc; Robert van der Boon, MSc; Abas Randjgari, MD; Carl Schultz, MD, PhD; Adriaan Moelker, MD, PhD; Robert-Jan van Geuns, MD, PhD; Fumiyuki Otsuka, MD; Patrick W. Serruys, MD, PhD; Renu Virmani, MD; Peter P. de Jaegere, MD, PhD

Background—Recent transcatheter aortic valve replacement studies have raised concerns about adverse cerebrovascular events. The etiopathology of the embolized material is currently unknown.

Methods and Results—A total of 40 patients underwent transcatheter aortic valve replacement with the use of a dual filter–based embolic protection device (Montage Dual Filter System, Claret Medical, Inc). Macroscopic material liberated during the transcatheter aortic valve replacement procedure was captured in the device filter baskets in 30 (75%) patients and sent for histopathologic analysis. The captured material varied in size from 0.15 to 4.0 mm. Amorphous calcified material (size, 0.55–1.8 mm) was identified in 5 patients (17%). In 8 patients (27%), the captured material (size, 0.25–4.0 mm) contained valve tissue composed of loose connective tissue (collagen and elastic fibers) with focal areas of myxoid stroma, with or without coverage by endothelial cells and intermixed with fibrin. In another 13 (43%) patients, collagenous tissue, which may represent elements of vessel wall and valvelike structures, was identified. In 9 patients (30%), thrombotic material was intermixed with neutrophils (size, 0.15–2.0 mm). Overall, thrombotic material was found in 52% of patients, and tissue fragments compatible with aortic valve leaflet or aortic wall origin were found in 52% (21/40) of patients.

Conclusions—Embolic debris traveling to the brain was captured in 75% of transcatheter aortic valve replacement procedures where a filter-based embolic protection device was used. The debris consisted of fibrin, or amorphous calcium and connective tissue derived most likely from either the native aortic valve leaflets or aortic wall. (Circulation. 2013;127:2194-2201.)

Key words: aortic valve ▪ intracranial embolism

After a decade of growing experience with transcatheter aortic valve replacement (TAVR), stroke has emerged as a vexing procedure-related complication. The 30-day stroke incidence in the randomized Placement of Aortic Transcatheter Valves (PARTNER) trial was 5.5% in the high-risk group, 6.7% in the inoperable patient cohort, and 3.2% in a weighted meta-analysis of 3519 TAVR patients. Half of these early cerebrovascular events occurred within 48 hours after the procedure and thus appear to be directly procedure related. TAVR currently requires the use of large-diameter devices (≥18F) and numerous accessory guidewires and catheters that interact with the aortic wall and aortic valve root, including the manipulation of calcified aortic valve leaflets. Diffusion-weighted MRI studies following TAVR demonstrate new subclinical ischemic brain lesions in up to 80% of patients. Through the use of transcranial Doppler, so-called high-intensity transient signals obtained during TAVR have shown that cerebral microembolization occurs predominantly during balloon valvuloplasty, transcatheter valve positioning, and implantation. Cerebral embolic protection devices have been suggested to provide protection to the brain from periprocedural embolization. Filter-based systems deployed along the extracranial cerebral arterial tree allow embolized debris to be captured and analyzed for composition. The etiology and pathology of debris that is embolizing to the brain during TAVR have not been previously reported and were impossible to determine without the capture and retrieval of the embolic debris. Elucidation of the etiology of this debris is important, because it may help improve preventive and therapeutic strategies. The aim of this study was to report on the histopathologic characteristics of the debris captured and retrieved with a dual filter–based cerebral embolic protection device during TAVR.

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Methods
Between December 2011 and September 2012, 40 patients judged to be at high operative risk by the institutional heart team (consisting...
of at least 1 cardiac surgeon, 1 interventional cardiologist, 1 imaging specialist, and 1 cardiac anesthesiologist) underwent TAVR for symptomatic severe aortic stenosis. One operator (N.M.v.M.) was trained in use of the Montage Dual Filter embolic protection device (EPD) (Claret Medical, Inc), a device that obtained CE Mark approval for use in TAVR on October 28, 2011. The Claret Montage EPD is a 6F compatible catheter delivered over a standard 0.014-in coronary guidewire and delivers 2 filters within 1 catheter to protect the cerebral vascular circulation (Figure 1). The first filter is deployed in the brachioce- phalic trunk to protect the right carotid artery, and the second filter is placed in the left common carotid artery (Figure 2). The conically shaped filters consist of a polyurethane film laser drilled with 140-μm holes and mounted onto Nitinol self-expanding wire frames.

The study population consisted of a total of 40 consecutive TAVR cases performed by the device-trained operator and using the Claret Montage EPD. Patient eligibility for EPD use required an appropriately sized right radial or brachial artery that could accommodate a 6F arterial sheath and compatible left common carotid artery (≥5 mm) and brachiocephalic artery (≥9 mm) diameters without significant stenosis (>70%) as determined by a multislice computed tomography scan. Clinical end points were prospectively collected and defined by using the Valve Academic Research Consortium definitions.11

TAVR Procedure and Debris Harvesting

All procedures were performed under general anesthesia. Patients were preloaded with dual antiplatelet therapy (aspirin and clopidogrel). A standardized anticoagulation regimen with heparin was initiated with a loading dose of 70 IU/kg aiming for an activated clotting time (ACT) between 250 and 300 seconds and with an ACT check at 30 minutes after the first bolus. Before the introduction of the large-bore (18F for arterial and 24F for apical access) TAVR access sheath and instrumentation of the aortic root, ascending aorta and aortic arch, the Claret Montage EPD was introduced through a 6F sheath placed in the right radial or right brachial artery, and the filters were deployed in the designated locations as described above (Figure 2). The aortic valve was cross with a straight wire, followed by an exchange for a stiffer support wire to allow for balloon valvuloplasty with the use of an undersized valvuloplasty balloon under rapid right ventricular pacing. Subsequently, the transcatheter heart valve, either the Medtronic CoreValve (Medtronic Inc, Minneapolis, MN) or the Edwards Sapien (Edwards Lifesciences Inc, CA) was implanted. After successful transcatheter heart valve implantation the Claret Montage EPD filters were retrieved and the device was removed. Outside the patient, the filters were exposed and examined for macroscopically visible debris (Figure 3). If present, the filters were cut, and the debris was passed through a 40-μm nylon cell strainer (BD Falcon filter), stored in a buffered formalin (4%) solution, and delivered to the Department of Pathology for analysis.

Histopathologic Assessment of Captured Debris

After measurement of the retrieved debris, the material was dehydrated and embedded in paraffin, 3- to 4-μm-thick sections were cut on a rotary microtome and routinely stained with hematoxylin and eosin and Movat pentachrome. Material of very small size (<0.25 mm) was processed following the Cellient procedure and stained with both Giemsa and hematoxylin and eosin.

To unravel the origin of the respective specimens, additional staining procedures were used: (1) CD34 and Factor VIII immunohistochemistry to identify capillaries and endothelial cells; (2) Fraser-Lendrum stain for fibrin detection; (3) Masson trichrome and Gomori to stain reticulin and collagen fibers; and (4) Elastica von Gieson to differentiate the aortic valve tissue from aortic wall or other vessel structures. The Department of Pathology of the Erasmus Medical Center, Rotterdam, the Netherlands, and the Cardiovascular Pathology Institute, Gaithersburg, MD, independently reviewed all prepared slides. The final pathology report of all slides was based on unanimous agreement.

Statistical Analysis

Continuous variables are presented as mean±standard deviation or median (quartile 1 to quartile 3); categorical variables are given as frequency (%).

Results

Baseline and Procedural Characteristics

A total of 40 patients underwent TAVR with embolic protection with the use of the Claret Montage EPD. Baseline and procedural characteristics are displayed in Tables 1 and 2. Mean age was 77±9 years, and 56% of the patients were male. History of cerebrovascular disease and atrial fibrillation was present in 6 (14%) and 8 (19%) patients, respectively. Approximately two thirds of patients were on antiplatelet therapy, and one fourth of patients were on anticoagulant therapy at baseline. The mean annulus size by multislice computed tomography was 24.7±2.2 mm. There was considerable aortic root calcification as illustrated by the mean Agatston score of 3018±1581. The transfemoral approach was the access strategy of first choice (90%), and

Figure 1. Claret Montage Dual Filter embolic protection device with curvable distal tip containing the 2 polyurethane filters.

Figure 2. The Claret Montage Dual Filter embolic protection device deployed in situ with the proximal filter (**) in the brachiocephalic trunk (black solid lines) and the distal filter (*) in the left common carotid artery (black dashed lines).
the Medtronic CoreValve system was used in 86% of cases (Table 2). Balloon postdilatation was performed in 30% of procedures. Mean per-procedural anticoagulation intensity was below the ACT target of 250 seconds. The Claret Montage EPD was introduced through a right radial arterial access (brachial access in 1 patient) and successfully deployed in all 40 patients. The introduction and deployment of the dual-filter catheter was safe and did not result in any complications.

Overall TAVR procedural success was obtained in all patients with the exception of 1 patient (Table 3). Clinical results included an all-cause 30-day mortality of 2.5% (1 patient) with an incidence of major vascular complications and life-threatening bleeding complications of 10% each. One patient had a transient ischemic attack on the sixth postoperative day. One patient who underwent TAVR through a left subclavian access had a ventricular perforation as a result of the stiff guidewire and eventually required sternotomy followed by suture closure of the lacerated left ventricular apex. This patient developed a periprocedural major stroke with an ischemic left occipital cerebral infarction corresponding to the left vertebral artery territory, as documented on multislice computed tomography brain scan, and died 13 days after the TAVR procedure.

**Histopathology**

All Claret Montage EPDs were successfully retrieved. Macroscopic debris was found in 1 or both filters in 75% (30/40) of cases and sent for histopathologic analysis (Figure 3). The captured material varied in size between 0.15 mm and 4.0 mm. The following 4 distinct histopathologic morphologies were identified (Table 4) in the 30 patients with captured debris:

1. Amorphous calcified material (diameter, 0.55–1.80 mm) was identified in 5 of 30 (17%) patients and represents the typical degenerative and calcified aortic valve leaflets (Figure 4A).
2. In 8 (27%) patients, the material recovered consisted of collagenous and proteoglycan matrix with elastic tissue (longest segment, 0.25–4.0 mm). The material was focally lined by endothelial cells resembling valve tissue, as is usually observed on the aortic surface above the calcified area (Figure 4B).

![Figure 3](http://circ.ahajournals.org/)

**Figure 3.** Debris captured in filters.

![Figure 4](http://circ.ahajournals.org/)

**Figure 4.** Histopathologic illustrations of captured debris retrieved from the Claret Montage Dual Filter. **A(i)**, The calcium fragment. **A(ii)**, The valve fragment. **B(i)**, Valve fragments, H&E stained; note proteoglycan-rich matrix. **B(ii)**, Elastic fibers and proteoglycans matrix are better appreciated on the Movat-stained section. **C**, Collagen fragments. **D**, Fragment of collagen and proteoglycan with thrombus (Movat stained). **E**, Thrombotic material consisting mostly of fibrin strands with trapped red blood cells and rare neutrophils. **F**, Valve tissue showing the presence of a nodule of Aranti. **G**, Necrotic material with thrombus, H&E stained. H&E indicates hematoxylin and eosin.
The typical hallmarks of vessel structures, ie, internal elastic laminae and smooth muscle cells in collagenous matrix, were not observed, and the usual histopathologic features of atherosclerosis (lipid-loaded macrophages: foamy cells, cholesterol crystals, intima smooth muscle cells in a proteoglycan matrix) were lacking. Figure 5 illustrates the gross pathology of aortic valve leaflets removed at surgery with one of the leaflets being decalcified, embedded in paraffin, and sectioned to show the histological appearance of a degenerative calcified aortic valve leaflet. Note the similarities between the calcified debris retrieved from the filter baskets and the stenotic aortic valve leaflet (Figure 5).

3. Pure collagenous material without any blood clot was seen in 4 (13%) cases.

4. Thrombotic material consisting of platelets, fibrin, and erythrocytes, with and without neutrophils (maximum diameter varied from 0.15 to 2.0 mm), were found in 21 (70%) cases. The thrombotic material was further differentiated into acute or chronic (organizing thrombus). Thrombus was classified as acute if it showed platelets and fibrin with interspersed red blood cells and acute inflammatory cells (neutrophils) but no interspersed spindle-shaped cells. Conversely, chronic thrombi showed the presence of spindle-shaped cells with or without macrophages that either lined the thrombus or infiltrated the thrombus, or had greater organization with matrix deposition interspersed between the fibrin. Of a total of 21 thrombi, 13 had features of acute thrombi, whereas 8 had features that fulfilled the definition of chronic thrombi (organizing thrombus).
Table 3. Clinical End Points According to the Valve Academic Research Consortium Definitions

<table>
<thead>
<tr>
<th>End Point</th>
<th>Overall n = 40</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-day or in-hospital death, n (%)</td>
<td></td>
</tr>
<tr>
<td>All-cause</td>
<td>1 (2.5)</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>1 (2.5)</td>
</tr>
<tr>
<td>Myocardial infarction, n (%)</td>
<td></td>
</tr>
<tr>
<td>Periprocedural (&lt;72 h)</td>
<td>0</td>
</tr>
<tr>
<td>Spontaneous (&gt;72 h)</td>
<td>0</td>
</tr>
<tr>
<td>Cerebrovascular complication, n (%)</td>
<td></td>
</tr>
<tr>
<td>Major stroke</td>
<td>1 (2.5)</td>
</tr>
<tr>
<td>Minor stroke</td>
<td>0</td>
</tr>
<tr>
<td>Transient ischemic attack</td>
<td>1 (2.5)</td>
</tr>
<tr>
<td>Vascular complication, n (%)</td>
<td></td>
</tr>
<tr>
<td>Major</td>
<td>4 (10.0)</td>
</tr>
<tr>
<td>Minor</td>
<td>2 (5.0)</td>
</tr>
<tr>
<td>Bleeding complication, n (%)</td>
<td></td>
</tr>
<tr>
<td>Life threatening</td>
<td>4 (10.0)</td>
</tr>
<tr>
<td>Major</td>
<td>3 (7.5)</td>
</tr>
<tr>
<td>Minor</td>
<td>6 (15.0)</td>
</tr>
<tr>
<td>Acute kidney injury, n (%)</td>
<td></td>
</tr>
<tr>
<td>Stage I</td>
<td>5 (12.5)</td>
</tr>
<tr>
<td>Stage II</td>
<td>1 (2.5)</td>
</tr>
<tr>
<td>Stage III</td>
<td>1 (2.5)</td>
</tr>
<tr>
<td>Composite safety end point, n (%)</td>
<td>9 (22.5)</td>
</tr>
</tbody>
</table>

Combined safety end point is the composite of all-cause mortality, major stroke, life-threatening bleeding, acute kidney injury-stage III, myocardial infarction, and repeat procedure for valve-related dysfunction.

Table 4. Distribution of Debris Found in the Overall Patient Cohort

<table>
<thead>
<tr>
<th>Histopathologic Characteristics</th>
<th>No. of Cases (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Calcium + valve tissue + thrombus</td>
<td>3</td>
</tr>
<tr>
<td>2 Calcium + valve tissue</td>
<td>2</td>
</tr>
<tr>
<td>3 Valve tissue</td>
<td>3</td>
</tr>
<tr>
<td>4 Thrombus</td>
<td>8</td>
</tr>
<tr>
<td>5 Thrombus + necrotic core</td>
<td>1</td>
</tr>
<tr>
<td>6 Thrombus + collagenous tissue</td>
<td>9</td>
</tr>
<tr>
<td>7 Collagenous tissue</td>
<td>4</td>
</tr>
</tbody>
</table>

Discussion

The principle findings of our histopathologic study on captured debris embolizing to the cerebrovascular circulation during TAVR are (Figure 7) as follows: (1) Macroscopic embolized debris was captured in 75% of patients (30/40); (2) in greater than one fourth (27%) of patients with captured debris (8/30), we found either amorphous calcium or distinct tissue likely originating from the degenerated aortic valve leaflets; (3) proteoglycan-rich and collagenous material, which may have come from either the aortic valve or the aortic wall, was identified in 43% of patients (13/30) with captured debris; (4) embolized tissue material (any combination of amorphous calcium with or without tissue from the aortic valve leaflet or aortic wall) was captured in over one half of all treated patients (21/40); and (5) thrombotic material was found in over one half of all cases (21/40).

Clinical and subclinical cerebrovascular events appear to be more frequent after TAVR than after surgical aortic valve replacement, and this may have immediate and long-term clinical implications.2,13–15 Brain MRI studies have established subclinical procedure-related cerebral diffusion-weighted imaging abnormalities in up to 80% of cases after TAVR, approximately double the rate that has been reported after surgical aortic valve replacement.6,7,14–18 Independent neurologi-
cal assessment of TAVR patients is not routinely performed, and, thus, some subtle neurological defects may go unrecognized; however, many emboli likely travel to clinically silent areas of the brain. These new cerebrovascular embolic events may not be trivial, because subclinical microinfarctions may be associated with neurocognitive decline and premature dementia.19 In addition, embolic events during the TAVR procedure may not result in clinically evident infarction for hours or even days until secondary changes result in thrombosis and actual infarction of brain tissue.

The histopathologic findings of the embolized material can be diverse but were heretofore unknown and subject to speculation. TAVR requires significant manipulation and instrumentration in the ascending aorta, aortic arch, descending aorta, and especially the aortic root. The use of guidewires, large-sized catheters, dilatation balloons, delivery systems, and the stented bioprosthesis may all promote thrombus formation through platelet aggregation and activation of the coagulation pathway. Foreign body material may also be released from these various percutaneous devices during the TAVR procedure. In addition, patients with severe aortic stenosis often have extensive atherosclerosis, including the presence of aortic arch plaques, and, because this condition is associated with cerebral embolization, these patients are at risk for mechanical dislodgment of plaque material during device passage.20 Finally, crossing a degenerative and heavily calcified aortic valve, performing a balloon aortic valvuloplasty, and introducing and deploying a bioprosthesis through the degenerative valve, all may result in detachment of debris from the aortic root (Figure 6).

Our study demonstrates that macroscopically visible debris can be captured in 75% of TAVR procedures (Figure 7). In 27% of patients (n=8) with captured debris, the material included amorphous calcified masses or connective tissue...
consistent with valve material without characteristic features of elastic arteries. In another 43% of patients (n=13), the captured debris contained collagenous proteoglycan-rich material, which may have either come from the aortic wall or from the valve, and one had necrotic core related to atherosclerosis. Therefore, we can conclude that the embolized material captured in 52% of the TAVR procedures (21/40) had been detached during instrumentation in and around the thoracic aorta and aortic root. These findings are concordant with a recent study on transcranial Doppler performed during TAVR that indicated that most high-intensity transient signals were generated during the valve implantation and deployment, and thus inherent to the TAVR procedure per se. We found evidence of thrombotic material in the EPD filters in 70% of subjects (n=21) with captured debris. Features of acute thrombus formation were detected in 13 patients mimicking suboptimal per-procedural anticoagulation. Conversely, chronic organizing thrombus was identified in 8 patients, which may suggest that it was attached to the vascular wall or aortic valve before the TAVR.

As TAVR technology shifts toward a lower risk and thus likely a younger patient population, the need to address and reduce cerebrovascular embolization becomes more urgent. Centers may therefore consider the adoption of an embolic...
protection strategy to reduce the cerebral burden of procedure-related valvular embolic debris.

The high prevalence of thrombotic material (52%, 21/40) also suggests a need for more reliable anticoagulation protocols, balancing between the risk for thromboembolic and bleeding complications. Variable patient response to heparin boluses is a well-known phenomenon and may result in subtherapeutic ACT levels. We measured suboptimal ACT levels (<250 milliseconds) at the 30-minute ACT check in 26 of 40 patients, which clearly justifies more meticulous anticoagulation protocols with closer ACT monitoring. Newer anticoagulants or more stringent periprocedural anticoagulation follow-up may reduce the frequency of captured thrombotic debris.

Limitations
This single-center descriptive study included a relatively low number of patients. Also, the Claret Montage EPD leaves the left vertebral artery uncovered, which may provide incomplete protection of the cerebral circulation. It is noteworthy that the 1 major stroke was localized in the left occipital cerebral lobe. Despite the fact we used a universally accepted standardized procedural anticoagulation protocol, low ACT levels at the 30-minute ACT check (in 26 patients) may have contributed to the frequency of thrombotic debris. Sampling error in the histopathologic analysis is possible, yet the results would probably be only more convincing if additional samples were included. Furthermore, all samples were analyzed by 2 experienced departments of pathology that independently analyzed all specimens. Our study is unique in that it is the first to identify the etiology of the debris that it is the first to identify the etiology of the debris that is captured in the overall population (n=40). The debris consisted of fibrin or amorphous calcified material and connective tissue derived from the native aortic valve leaflets and the aorta. This study provides the first documentation of the high frequency, large size, and varied content of embolic debris liberated during TAVR that can be captured before reaching the brain with the use of an EPD.

Conclusion
Embolic debris traveling to the brain was captured and retrieved in three fourths of TAVR procedures by deploying a dedicated filter-based EPD. The debris consisted of fibrin or amorphous calcified material and connective tissue derived from the native aortic valve leaflets and the aorta. This study provides the first documentation of the high frequency, large size, and varied content of embolic debris liberated during TAVR that can be captured before reaching the brain with the use of an EPD.

Disclosures
None.

References
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**Clinical Perspective**

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Circulation. 2013;127:2194-2201; originally published online May 7, 2013; doi: 10.1161/CIRCULATIONAHA.112.001091
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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