Immunohistochemical Analysis of Thrombi Retrieved During Treatment of Acute Ischemic Stroke

Does Stent-Retriever Cause Intimal Damage?

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Background and Purpose—To provide evidence whether mechanical thrombectomy with stent-retrievers in the treatment of acute ischemic stroke causes intimal damage.

Methods—This study analyzed thrombi retrieved by mechanical thrombectomy from cerebral arteries in 48 consecutive patients with acute ischemic stroke using CD34 antibodies.

Results—Of 48 thrombi analyzed, CD34-positive cells were absent in 20, present as isolated cells in 21, and found in clusters in 7 thrombi. We did not find any subendothelial vessel wall structures.

Conclusions—Our findings suggest that mechanical thrombectomy with stent-retrievers does not cause relevant intimal damage in acute ischemic stroke treatment.

Clinical Trial Registration—URL: http://www.germanctr.de. Unique identifier: DRKS00004695 (Stroke. 2013;44:00-00.)

Key Words: immunohistochemistry ■ ischemic stroke ■ thrombectomy ■ thrombus

Thrombolysis with intravenous administration of recombinant tissue-type plasminogen activator has proven effective in randomized controlled trials. However, in large-vessel occlusion with high thrombus burden, poor recanalization rates with limited patient outcome have been reported. Most recently, a new class of mechanical thrombectomy devices has emerged with reported recanalization rates ranging from 67% to 100%. However, the stress to the vessel wall related to stent-retrieval seems important. During retraction of the stent-retriever, the vascular anatomy may be distorted and considerable vasospasm of the target vessel and along the course of the stent-retriever are observed on a regular basis. To date no histopathologic data on the effect of mechanical thrombectomy with stent-retrievers on the intima in men are available.

In this study, we analyzed whether withdrawal of the unfolded stent by mechanical force to perform thrombectomy in acute ischemic stroke causes intimal injury. We, therefore, analyzed thrombi retrieved from acutely occluded vessels with stent-retrievers, for occurrence of denuded endothelial cells and subendothelial connective tissue.

Methods

Patient Selection

This study was approved by the institutional review board of the University Hospital Freiburg. All interventional procedures were in accordance with institutional guidelines.

We included 48 patients admitted for acute ischemic stroke in which we were able to retrieve thrombus material by means of mechanical thrombectomy with stent-retrievers. Routine baseline investigations included neurological and physical examination, assessment of National Institutes of Health Stroke Scale (NIHSS), brain imaging with either computed tomography or MRI, and vascular imaging with either computed tomography angiography or MR angiography. The presumed ischemic stroke mechanism was determined with use of the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) criteria by the stroke neurologist. Patient neurological status was scored with a further NIHSS at discharge. Good neurological outcome was defined as a NIHSS score of 0 to 4 or a NIHSS score improvement of >9 points.

Thrombus Retrieval Procedure

The thrombectomy procedure was performed as described elsewhere. The Solitaire FR (Solitaire 4 mm, Covidien) was used in 39 patients, 9 patients were treated with the Aperio device (Aperio 4.5 mm, Acandis). Recanalization was classified according to the Thrombosis in Cerebral Infarction (TICI) criteria. TICI 2b and 3 were considered a successful recanalization. A case example of a successful mechanical thrombectomy is displayed in Figure 1.

Processing of Thrombi and Analysis

Thrombi were mostly retrieved in multiple fragments, completely removed from the stent-retrievers, and fixed in phosphate buffered formalin within the angiosuite. Thrombus material was embedded in paraffin, sectioned at 4-μm thickness and stained with hematoxylin and eosin, Prussian-blue, Elastica-van-Gieson, Kossa, and Periodic acid-Schiff reaction. All thrombi specimens were in addition tested immunohistochemically for CD34 (monoclonal, class II, clone QBEnd10, DAKO) to detect and quantify endothelial cells.
example for positive CD34 immunohistochemistry is shown in Figure 2. The CD34 assays were performed retrospectively. The slides were analyzed by a board-certified neuropathologist (S.D.) without knowledge of the clinical findings. The degree of underlying intimal damage was categorized into 3 grades: (1) absence of endothelial cells: intimal damage unlikely; (2) single or clustered endothelial cells: minor intimal damage cannot be excluded; (3) endothelial cells and subendothelial connective tissue: relevant intimal damage confirmed.

Results

Patient Data

From July 2011 to October 2012, 48 consecutive patients with acute ischemic stroke were included into the study. There were 29 men and 19 women, the mean age was 67 years (range, 43–91 years; SD, 12). Mean NIHSS score on admission was 19 (range, 7–34; SD, 5). Twenty seven patients received intravenous recombinant tissue-type plasminogen activator before the endovascular intervention. The mean time interval from stroke onset to start of cerebral angiography was 4 hours 8 minutes (range, 1 hour 53 minutes to 8 hours 6 minutes; SD, 1 hour 53 minutes).

Angiographic and Clinical Outcome

Angiography demonstrated combined occlusion of internal carotid artery (ICA) and M1 in 25 patients, occlusion of M1 in 18, and the basilar artery in 5. Percutaneous transluminal angioplasty and stenting were performed before stent-retrieval in 12 patients with an underlying high-grade stenosis of the ICA and 1 patient with a high-grade stenosis of the proximal vertebral artery. TICI 2b/3 results were achieved in 35/48 patients (73%). The average number of passes required was 2 (range, 1–5). Vasospasm of the target vessel and along the course of the stent-retriever was observed in 20/48 patients. Dissections of the ICA occurred in 4 patients. These were limited to the extracranial ICA and related to either the balloon catheter or ICA stenting. Mean time from symptom onset to successful recanalization/end of procedure was 5 hours 43 minutes (range, 3 hours 7 minutes to 12 hours 23 minutes; SD, 1 hour 54 minutes). Presumed origins of thrombi were athero-embolic in 18, cardio-embolic in 29, and remained cryptogenic in 1. On discharge, 18/48 patients (38%) had a good clinical outcome.

Histopathologic Outcome

Of the retrieved clots, 13 were classified as fibrin dominant, 15 red blood cell dominant, and 20 were mixed. CD34-positive endothelial cells were absent in 20 thrombi (grade I) and present in 28 (grade II). In 21 thrombi those endothelial cells were isolated, in 17 of these CD34-positive cells were considered to represent surface endothelialization. Clustered endothelial cells of >1 cell were found in 7 cases. We did not detect any associated subendothelial connective tissue (grade III). Polymorphonuclear cell infiltration was present in 33 thrombi (in 3 red, 11 white, and 19 mixed thrombi) of which 30 thrombi (91%) were either white or mixed subtype.

In a univariate analysis, the presence of clustered endothelial cells was not associated with a poorer clinical outcome, the occurrence of vasospasm, or a TICI score 0-2a. Moreover, there was no significant association between thrombus histology (red, white, and mixed) and procedure time for mechanical thrombectomy, TICI score 0-2a, and poor clinical outcome.

Discussion

This study provides systematic histological analysis of thrombi retrieved from 48 consecutive patients with acute ischemic stroke undergoing mechanical thrombectomy with stent-retriever. Retrieved thrombi varied between fibrin dominant, red blood cell dominant, and mixed thrombi. Our observations on the great variety in overall appearances of retrieved thrombi corroborate earlier findings reported in the
In addition, we were able to confirm findings by Marder et al that thrombus histology does not seem to be of predictive value for successful mechanical thrombectomy.

The aim of this study was to analyze the potentially damaging effect of mechanical thrombectomy with stent-retrievers on the target vessel intima. In our histological assessments, we did not find any subendothelial components of the vessel intima within analyzed specimens. Although the forces applied to the target vessels and the vessels along the course of the stent-retriever were sufficient to induce vasospasm in 20/48 patients, we did not find histopathologic evidence of relevant intimal damage. Single endothelial cells or small clusters of endothelial cells might belong to the physiological turnover of the endothelium. In addition, these endothelial cells might correspond to a surface endothelialization—a sign of beginning thrombus organization.

Nogueira et al reported on severe disruption of the intima observed in a preclinical study of a thrombectomy device in 2 different animal models of arterial thrombo-occlusive disease. Yet, the histopathologic analysis showed no hemorrhage of media or adventitia. These findings might not lead to long-term vessel damage. The authors did not find any microscopic evidence of dissection, pseudoaneurysm formation, or inflammation in the vessel wall.

In the literature on various different thrombectomy devices, vasospasm is regularly reported. Yet dissections or subarachnoid hemorrhage associated with the procedure remains exceptional. To date no histopathologic data on the effect of thrombectomy with stent-retrievers on the intima in men are available. Kurre et al analyzed the long-term side effects of mechanical thrombectomy with stent-retrievers on intracranial vessels in 116 patients. On follow-up, digital subtraction angiography target vessel occlusion was present in 1, and de novo stenosis occurred in 4 vessel segments.

As a limitation, our study was conducted in a single center. CD34 is not specific for endothelial cells and may be expressed on early hematopoietic cells as well. Two different stent-retrievers were used in this trial.

**Conclusion**

Our findings suggest that mechanical thrombectomy with stent-retrievers does not cause relevant intimal damage in acute ischemic stroke treatment.

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**Disclosures**

Dr. Singh has received a grant from the European Society of Radiology for a research stay at the Department of Neuroradiology, University Hospital Freiburg, Germany. The other authors have no conflicts to report.

**References**

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