Immunohistochemical Analysis of Debris Captured by Filter-Type Distal Embolic Protection Devices for Carotid Artery Stenting

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Background: Little is known about the micro-debris captured in filter-type distal embolic protection devices (EPD) used for carotid stenting (CAS). This study aimed to determine the histological and immunohistochemical characteristics of such debris by using a new liquid-based cytology (LBC) technique. *Methods:* Fifteen patients who underwent CAS using a filter-type distal EPD (FilterWire EZ; Boston Scientific, Marlborough, MA, USA) were included in the study. After gross inspection of each recovered filter device, micro-debris were collected using a new LBC technique (SurePath; TriPath Imaging, Inc., Burlington, NC). Histological and immunohistochemical analysis of the recovered debris was performed. The pre- and postoperative brain magnetic resonance imaging and neurological status of each patient were also reviewed. *Results:* No patient developed ipsilateral symptomatic stroke due to a thromboembolic event. All 15 patients (100%) had microscopically identifiable debris in the filters, whereas gross inspection detected visible debris only in 5 patients (33.3%). Histological analysis revealed various types of structural components in an advanced atheromatous plaque, including fragments of fibrous cap, calcified plaque, smooth muscle cells, and necrotic tissue fragment infiltrated with monocytes and macrophages. *Conclusions:* Filter-type EPDs may contribute to reducing the risk of CAS-related embolic events by capturing micro-debris even when gross inspection of the recovered filter shows no visible debris in the device. **Key Words:** Carotid artery stenting—embolic protection device—micro-debris—histological analysis.

Introduction

Studies suggest that post-procedural ipsilateral stroke, most likely caused by a thromboembolic event during stent deployment, remains a serious complication of carotid artery stenting (CAS).[1-4] Filter-type embolic protection devices (EPDs) are designed to reduce the risk of distal embolic event during CAS procedures. Several clinical studies have shown that the use of EPDs is associated with lower risk of embolic events.[4-6] However, little is known about the micro-debris captured in these devices, partially because a layer of blood in the recovered filter often obscures the visibility of the miniscule contents.

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Liquid-based cytology (LBC) test is a cytology technique used in gynecology to detect cellular components in Pap smear samples, which are frequently replete with red blood cells.[7] A new LBC test called "SurePath" (TriPath Imaging, Inc., Burlington, NC) is known to have a higher detection rate than conventional LBC tests, mainly owing to its unique density-gradient centrifugation process.[8-10]

In this study, micro-debris captured in filter-type EPDs used in CAS were recovered using SurePath and underwent thorough histological and immunohistochemical analysis. The clinical characteristic and image findings of each treated patient were also reviewed.

Methods

The CAS Procedure

From March 2013 to October 2014, 29 patients with carotid stenosis (4 symptomatic) underwent the CAS procedure. Among them, 15 patients who were treated using FilterWire EZ (FWEZ; Boston Scientific, Marlborough, MA) were selected for this study.

All of the patients were started on dual antiplatelet therapy with the combination of 75 mg clopidogrel per day and 100 mg aspirin per day. If the patient was a poor or nonresponder to clopidogrel, the combination of 100 mg of aspirin and 200 mg of cilostazol per day was selected. Dual antiplatelet therapy was started at least 7 days before the procedure.

All procedures were performed via femoral access under local anesthesia. Systemic heparinization was performed with the goal of maintaining the activating clotting time within 250-300 seconds during the procedure.

A 6 Fr shuttle sheath was advanced via the femoral artery and placed at the treatment side of the common carotid artery. After the stenotic lesion was crossed with a FWEZ, the filter device was deployed at the distal segment of the cervical internal carotid artery. Predilatation was performed using an angioplasty balloon (Sterling; Boston Scientific, Natick, MA), and an appropriate sized Carotid WALLSTENT (Boston Scientific) was deployed over the FWEZ. Post-dilation was performed using the same angioplasty balloon. After confirming sufficient dilatation of stenotic lesion in the post-procedure angiogram, FWEZ was re-sheathed into a dedicated recovery sheath.

The Institutional Review Board of the Jikei University School of Medicine approved the study protocol.

Evaluation of Post-Procedure Imaging and Clinical Findings

For the pre- and post-procedure imaging analysis, all of the patients underwent brain magnetic resonance imaging (MRI) (1.5 T). The sequences included diffusion-weighted image (DWI), fluid-attenuated inversion recovery, susceptibility-weighted imaging, and magnetic resonance angiography. Any high-intensity signal detected in the post-procedure DWI was considered a thromboembolic complication and was recorded. Neurological examination was performed immediately after the procedure and daily during the hospitalization. A modified Rankin scale on the day of discharge was also recorded as a clinical outcome.

Debris Analysis

Immediately after recovery of the FWEZ from the guiding catheter, the delivery wire near the device was cut with sterilized scissors and the filter was carefully submerged in the SurePath preservative fluid. Rinsing or flushing the filter with saline was avoided to prevent the loss of micro-debris.

The preservative was composed of multiple alcohols (ethanol 21.7%, methanol 1.2%, and isopropanol 1.1%) for the osmotic hemolysis of the red blood cells and fixation and rinsing of the cellular components. Each sample was then mixed by vortexing, and the debris were separated from the supernatant by centrifugation. The supernatant was discarded, and for the next enrichment step, centrifugal sedimentation through 3 mL of a hemolytic fixative, CytoRich Red Preservative (TriPath Imaging, Inc.), was performed. After centrifugation, the pelleted micro-debris were re-suspended with 500 µmL of distilled water, mixed, and transferred to a PrepStain Settling Chamber (TriPath Imaging, Inc.) mounted on a SurePath PreCoat slide. The debris were then sedimented by gravity for 10 minutes. The settling chamber was used to deposit a thin layer of micro-debris concentrated within a circular area (φ13 mm) on the glass slides, which were coated with poly-L-lysine (beta-helix structure). After rinsing the slide using 95% ethanol, the sample was fixed with 100% ethanol.

Each prepared slide glass was then stained by multiple staining, including hematoxylin and eosin (H&E), Masson trichrome, elastic Verhoeff-van Gieson (EVG), Papanicolaou, and Kossa. Immunohistochemical analysis was performed using alfa smooth muscle actin (αSMA) and CD68. The debris, presented within a 13-mm-diameter circle on the slide glass, were examined under a microscope by a trained neuropathologist and neurosurgeons.

Because of the limited amount of recovered tissue sample, H&E staining was prioritized and the other types of staining, including immunohistochemical analysis, were selected based on the types of tissue fragment observed in the primary staining. For instance, if a sample showed preponderance of noncellular component, stains focusing on the extracellular matrix (e.g., Masson trichrome and EVG) were selected. For samples with rich cellular component, immunohistochemical analysis e.g. CD68 staining and αSMA staining were performed.