CLINICAL STUDY

# Endovascular Biopsy and Endothelial Cell Gene Expression Analysis of Dialysis Arteriovenous Fistulas: A Feasibility Study

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## ABSTRACT

**Purpose:** To demonstrate feasibility of endothelial cell (EC) biopsy from dialysis arteriovenous fistulas (AVFs) with the use of guidewires and to characterize gene expression differences between ECs from stenotic and nonstenotic outflow vein segments.

**Materials and Methods:** Nine consecutive patients undergoing fistulography for AVF dysfunction from June to August 2016 were enrolled. ECs were biopsied with the use of guidewires from venous outflow stenoses and control outflow veins central to the stenoses. ECs were sorted with the use of flow cytometry, and the Fluidigm Biomark HD system was used for single-cell quantitative polymerase chain reaction (qPCR) analysis of gene expression. Forty-eight genes were assessed and were selected based on different cellular functions and previous literature. Linear mixed models (LMMs) were used to identify differential gene expression between the groups, and self-organizing maps (SOMs) were used to identify cell clusters based on gene coexpression profiles.

**Results:** A total of 219 and 213 ECs were sampled from venous outflow stenoses and control vein segments, respectively. There were no immediate biopsy-related complications. Forty-eight cells per patient were sorted for qPCR analysis. LMM identified 7 genes with different levels of expression at stenotic segments ($P < .05$), including *AGTR−2, HMOX−2, MTHFR, SERPINC−1, SERPINE−1, SMAD−4*, and *VWF*. SOM analysis identified 4 cell clusters with unique gene expression profiles, each containing stenotic and control ECs.

**Conclusions:** EC biopsy from dialysis AVFs with the use of guidewires is feasible. Gene expression data suggest that genes involved in multiple cellular functions are dysregulated in stenotic areas. SOMs identified 4 unique clusters of cells, indicating EC phenotypic heterogeneity in outflow veins.

## ABBREVIATIONS

AVF = arteriovenous fistula, EC = endothelial cell, LMM = linear mixed models, qPCR = quantitative polymerase chain reaction, SOM = self-organizing map

Venous outflow stenosis because of neointimal hyperplasia and medial fibrosis accounts for the majority of dialysis arteriovenous fistulas (AVFs) requiring intervention to maintain patency (1). The histologic hallmark of neointimal hyperplasia is smooth muscle cell and myofibroblast migration and proliferation within the neointima (2). The mechanisms mitigating this process are not well understood, with current knowledge largely based on immunohistochemical analysis of resected surgical specimens (3) and genetic analysis of in vitro (4) and nonhuman animal models (5–8).

The lack of a safe and reliable means for cellular analysis of AVFs in humans has limited the genetic characterization of AVF failure. Consequently, the ability to predict AVF

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Table E1 can be found by accessing the online version of this article on *www.jvir.org* and clicking on the Supplemental Material tab.

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**EDITORS' RESEARCH HIGHLIGHTS**

- The authors demonstrated that a 1.5-mm-diameter J-type guidewire successfully harvested endothelial cells (ECs) from within stenotic dialysis AV fistulas for gene characterization.
- A number of dysregulated genes, ones not found in normal fistula segments, were identified. This suggests that heterogeneity of EC phenotypes at specific vessel segments may play a role in stenosis formation. The findings also suggested that diabetes was a possible modifier of endothelial gene function.

failure and identify pharmacologic therapies that may prevent or slow venous outflow stenosis are limited. To address this limitation, the present study expands on previous endovascular techniques (9–14) for the targeted biopsy of endothelial cells (ECs) in vivo in dialysis AVFs with the use of endovascular devices. The objectives of this study were: (*i*) to demonstrate feasibility of EC biopsy in dialysis AVFs with the use of guidewires; (*ii*) to characterize gene expressivity differences between ECs from stenotic and nonstenotic outflow vein segments; and (*iii*) to develop an analytic technique using linear mixed models (LMMs) to conduct unsupervised machine-learning statistical analysis of single-cell gene expression.

## MATERIALS AND METHODS

The study protocol was approved by the Institutional Review Board.

### Patients

Ten consecutive patients on hemodialysis referred to the interventional radiology service for fistulography from June to August 2016 were enrolled and consented to participate in this study. One patient was excluded from the final analysis owing to the presence of an AV graft. Inclusion criteria included end-stage renal disease, a dialysis AVF with a peripheral venous outflow stenosis, and consent for tissue analysis. Patient demographics and clinical data were obtained from the most recent primary care clinic notes in the electronic medical record (**Table**). Three patients were male. The mean patient age was 61 years (± 11 years). Seven patients had hypertension, 5 were diabetic, 1 was a current smoker, and 4 were past smokers. The mean BMI was 28.8 kg/m$^2$ (± 10.9). Severity of stenosis was quantified from images saved in our Picture Archiving and Communication System. Percentage of stenosis was calculated by dividing the diameter of the stenosis by the mean of measurements taken from nonstenotic vessel segments immediately proximal and distal to the stenosis.

### Endothelial Biopsy

A 6-F sheath (Cordis, Milpitas, California) was placed in the peripheral venous outflow and fistulography was performed. After identification of a peripheral venous outflow stenosis, a 0.035-inch Glidewire (Terumo, Tokyo, Japan) was advanced central to the stenosis. The stenosis was then crossed with a 5-F angled catheter (Kumpe; Cook Medical, Bloomington, Indiana) and the Glidewire was removed. A 0.035-inch "J"-tipped guidewire (Rosen; Cook Medical) was then advanced to the distal aspect of the catheter (**Fig 1**). The catheter was withdrawn to expose the distal 1 cm of the guidewire, which was then advanced and retracted within the stenosis at 1-cm intervals ~20 times. The "J" tip of the wire opened relative to the degree of the stenosis. The guidewire was removed and the distal 5 cm was cut and immediately placed in a 50-mL tube (Falcon; Thermo Fisher Scientific, Waltham, Massachusetts) containing 10 mL EC dissociation buffer (Gibco; Thermo Fisher Scientific, Waltham, Massachusetts). Control samples from angiographically normal venous outflow vein central to the stenosis were obtained in a similar fashion. Care was taken during both experimental and control biopsies to ensure contact between the wire and the target vessel only. The sampling wires were otherwise sheathed within the delivery catheter.

### Endothelial Sorting

Endothelial cells were sorted according to the protocol described in detail by Sun et al (10). Forty-eight cells were sorted from each patient. Briefly, after dislodging cells by vortex and centrifuge, the sampled cells were labeled with 4 EC-specific antibodies (CD31, CD34, CD105, CD146), 1 myeloid-specific antibody (CD11b), and 1 platelet-specific antibody (CD42) to identify and sort single ECs. ECs were sorted based on CD31+, CD34+, CD105+, CD146+, CD11b−, and CD42−. Aria II fluorescence-activated cell sorting (FACS; BD Biosciences, Franklin Lakes, New Jersey) with a 100 nm nozzle in single-cell sort mode was used.

### Reverse Transcription and cDNA Preamplification

Each single EC was sorted directly into 1 well of a 96-well plate with 9 μL reverse transcription–specific target amplification (RT-STA) buffer. The RT-STA buffer contained 5 μL Cellsdirect 2× Reaction Mix (Thermo Fisher Scientific), 0.2 μL SuperScript III RT Platinum Taq Mix (Life Technologies, Carlsbad, California), 2.8 μL nuclease-free water, and 1 μL 10× primer mixture (Fluidigm, San Francisco, California).

The primer pairs were selected based on previous nonhuman animal and in vitro studies identifying gene pathways that potentially affect AVF failure, including transforming growth factor (TGF) β–dependent pathways and pathways involved in vascular injury, repair, and thrombosis. Components of these genetic pathways with available drug inhibitors or promoters were also selected (**Table E1** [on the article's Supplemental Material page at *www.jvir.org*]) (5,8,10). Primers were custom designed by Fluidigm to cross introns and avoid amplifying genomic