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ORIGINAL CLINICAL SCIENCE

Vascular inflammation and abnormal aortic histomorphometry in patients after pulsatile- and continuous-flow left ventricular assist device placement

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BACKGROUND: Left ventricular assist devices are increasingly being used in patients with advanced heart failure as both destination therapy and bridge to transplant. We aimed to identify histomorphometric, structural and inflammatory changes after pulsatile- and continuous-flow left ventricular assist device placement.

METHODS: Clinical and echocardiographic data were collected from medical records. Aortic wall diameter, cellularity and inflammation were assessed by immunohistochemistry on aortic tissue collected at left ventricular assist device placement and at explantation during heart transplantation. Expression of adhesion molecules was quantified by Western blot.

RESULTS: Decellularization of the aortic tunica media was observed in patients receiving continuous-flow support. Both device types showed an increased inflammatory response after left ventricular assist device placement with variable T-cell and macrophage accumulations and increased expression of vascular E-selectin, ICAM and VCAM in the aortic wall.

CONCLUSIONS: Left ventricular assist device implantation is associated with distinct vascular derangements with development of vascular inflammation. These changes are pronounced in patients on continuous-flow left ventricular assist and associated with aortic media decellularization. The present findings help to explain the progressive aortic root dilation and vascular dysfunction in patients after continuous-flow device placement.

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Left ventricular assist devices (LVADs) are increasingly being used in end-stage heart failure as a bridge to transplant as well as destination therapy. Currently, continuous-flow

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LVADs (cfLVADs) are the preferred device type because of their smaller size, long-term durability, energy efficiency, decreased thrombogenicity, lower risk of infection and decreased surgical trauma compared with pulsatile-flow LVADs (pLVADs).[1,2] Furthermore, earlier studies have demonstrated that cfLVADs are associated with increased survival during LVAD support in patients awaiting heart transplantation.[2,3] Nevertheless, use of the current generation of continuous-flow devices is associated with significant bleeding rates, particularly gastrointestinal bleeding due to arteriovenous malformations,[4] as well as von Willebrand factor (vWF) deficiency,[5] cerebrovascular events,[6] drive-line and pocket infections,[7] development of aortic insufficiency[8,9] and high rates of device thrombosis.[10,11]

Current evidence suggests that pulsatile flow is not needed for systemic end-organ perfusion.[1,12–14] However, the long-term implications of non-pulsatile blood flow on the vasculature, particularly the aorta, remain unclear, and it has been reported that vascular compliance and distensibility are altered during cfLVAD support.[15] The aim of this study was to compare vascular histomorphometric, molecular and physiologic changes in patients supported by pulsatile- vs continuous-flow LVADs.

Methods

We retrospectively analyzed 27 patients undergoing LVAD implantation as bridge to transplant followed by subsequent LVAD explantation for cardiac transplantation at Columbia University Medical Center between February 2005 and November 2010. This included 15 pLVAD and 12 cfLVAD patients. The pLVAD group received the HeartMate XVE device (Thoratec Corp, Pleasanton, CA) and the cfLVAD group received the HeartMate II device (Thoratec Corp.). All patients in the study had New York Heart Association Stage IV heart failure (HF) at the time of LVAD implantation. Aortic tissue was collected at the time of LVAD placement (at the site of the outflow graft–aorta anastomosis) and at the time of LVAD explantation (at the site of end-to-end aortic anastomosis) during cardiac transplantation. Samples were immediately snap frozen, placed in liquid nitrogen for transport, and stored at −80°C until final analysis. Clinical and laboratory characteristics were collected from electronic medical records. Of the cohort, only 2 of the LVAD implant and explant samples were paired.

The present study was approved by the institutional review board of Columbia University Medical Center. All patients provided written informed consent before inclusion in the study.

Echocardiography

Echocardiography was used to determine the dimensions of the proximal aortic root and identify any degree of aortic insufficiency (Philips Healthcare Corp., Andover, MA). A standard comprehensive M-mode, 2D echocardiogram and Doppler studies were performed on each patient before surgery.

Histomorphometric analysis

Formalin-fixed aortic tissue samples were embedded in paraffin and cut into 5-μm-thick sections. Hematoxylin-and-eosin staining (H&E; Sigma) was carried out to determine cellularity and wall thickness of the aorta. Image J software (NIH) was used to quantify the number of cells for the tunica intima, media and adventitia of the aortic wall (100× magnification, number of cells/mm^2). In addition, Image J software was used to quantify aortic wall thickness (40× magnification, average of 3 measurements).

To prepare the sections for immunohistochemistry, the slides were deparaffinized and rehydrated. Antigen retrieval was completed with 10 mmol/liter sodium citrate (heated), pH 6.0, for 30 minutes. Sections were blocked in 6% normal serum and incubated with primary antibodies for 1 hour. CD3 (MCA1477; AbD Serotec) and CD68 (ab955; Abcam) antibodies were used for detection the presence of T cells and macrophages, respectively. Endogenous horseradish peroxidase (HRP) activity was reduced by incubating the sections in a 3% peroxidase solution for 10 minutes. Sections were then incubated with the appropriate biotinylated secondary antibody for 30 minutes. Secondary staining was carried out using the VectaStain ABC kit (Vector Laboratories, Inc., Burlingame, CA). Sections were then developed using immPACT NOVA-red chromogen substrate (Vector Laboratories). Sections were counterstained with hematoxylin and dehydrated before mounting the coverslip. Image J software was used to quantify the number of T cells and macrophages (100× magnification, number of cells/mm^2).

Protein analysis

Tissue homogenates were processed in 200 to 300 μl of precipitation assay buffer (Sigma) supplemented with phosphatase and protease inhibitors (Roche, Indianapolis, IN). Protein extraction was performed using standard techniques. Protein extracts were resolved on 4% to 15% sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS-PAGE) reducing gels (Bio-Rad) transferred to polyvinylidene difluoride membranes (Bio-Rad), and probed with E-selectin (3631-100; Santa Cruz Biotechnology), intracellular adhesion molecule (ICAM; SC-1511; Cell Signaling), vascular cell adhesion molecule (VCAM; AC-1504; Santa Cruz Biotechnology) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH; 3683S; Cell Signaling Technologies) antibodies. Membranes were washed in Tris-buffered saline and incubated with the appropriate secondary antibody conjugated to HRP. Detection was carried out using an ECL Western Blotting System kit (Thermo Scientific). All Western blots were analyzed using the Molecular Imager Gel Doc XR+ system with IMAGELAB software (Bio-Rad).

Statistical analysis

GraphPad Prism 5 was used for all statistical analyses. Results for unpaired data were analyzed using 2-tailed unpaired Student's *t*-tests. Data are expressed as mean ± standard deviation. $p < 0.05$ was considered significant.

Results

Baseline characteristics

We retrospectively analyzed 27 patients undergoing LVAD implantation as bridge to transplant followed by LVAD explantation for HTx. This included 15 pLVAD patients (9 patients at implantation and 6 patients at explantation) and 12 cfLVAD patients (7 patients at implantation and 5 patients at explantation). Descriptive characteristics are