Left-Ventricular Assist Device Impact on Aortic Valve Mechanics, Proteomics and Ultrastructure

Elizabeth H. Stephens, MD, PhD, Jiho Han, BS, Emma A. Trawick, BA, Elena S. Di Martino, PhD, Hemanth Akkiraju, PhD, Lewis M. Brown, PhD, Jennifer P. Connell, PhD, K. Jane Grande-Allen, PhD, Gordana Vunjak-Novakovic, PhD, and Hiroo Takayama, MD, PhD

Division of Cardiac, Thoracic and Vascular Surgery, Columbia University Medical Center, New York, New York; Department of Biomedical Engineering, Columbia University, New York, New York; Schulich School of Engineering and Libin Cardiovascular Institute, University of Calgary, Calgary, Alberta, Canada; Quantitative Proteomics and Metabolomics Center and Department of Biological Sciences, Columbia University, New York, New York; Department of Bioengineering, Rice University, Houston, Texas; and Department of Medicine, Columbia University Medical Center, New York, New York

Background. **Aortic regurgitation is a prevalent, detrimental complication of left ventricular assist devices (LVADs). The altered hemodynamics of LVADs results in aortic valves (AVs) having distinct mechanical stimulation. Our hypothesis was that the altered AV hemodynamics modulates the valve cells and matrix, resulting in changes in valvular mechanical properties that then can lead to regurgitation.**

Methods. **AVs were collected from 16 LVAD and 6 non-LVAD patients at time of heart transplant. Standard demographic and preoperative data were collected and comparisons between the two groups were calculated using standard statistical methods. Samples were analyzed using biaxial mechanical tensile testing, mass spectrometry-based proteomics, and transmission electron microscopy to assess ultrastructure.**

Results. **The maximum circumferential leaflet strain in LVAD patients was less than in non-LVAD patients (0.35 ± 0.10MPa versus 0.52 ± 0.18 MPa, $p = 0.03$) with a trend of reduced radial strain ($p = 0.06$) and a tendency for the radial strain to decrease with increasing LVAD duration ($p = 0.063$). Numerous proteins associated with actin and myosin, immune signaling and oxidative stress, and transforming growth factor β were increased in LVAD patients. Ultrastructural analysis showed a trend of increased fiber diameter in LVAD patients (46.2 ± 7.2 nm versus 45.1 ± 6.9 nm, $p = 0.10$), but no difference in fiber density.**

Conclusions. **AVs in LVAD patients showed decreased compliance and increased expression of numerous proteins related to valve activation and injury compared to non-LVAD patients. Further knowledge of AV changes leading to regurgitation in LVAD patients and the pathways by which they occur may provide an opportunity for interventions to prevent and/or reverse this detrimental complication.**

(Ann Thorac Surg 2017;■:■–■)

Aortic regurgitation (AR) has been increasingly recognized as a relatively common, detrimental complication of left ventricular assist device (LVAD) support leading to decreased pump efficiency and worsening heart failure. A recent study showed that the incidence of de novo moderate or greater AR after LVAD is 22% at 1 year and ~40% at 3 years, with one third of these patients requiring surgical intervention [1].

LVADs are known to alter the aortic valve's (AV's) hemodynamic environment, including increasing transvalvular pressure, decreasing aortic pulsatility, and disrupting the vortices in the sinuses of valsalva [2]. Additionally, in many patients the AV remains closed or intermittently partially open.

Advances in our understanding of valve mechanobiology have shown that valve cells and the composition they produce respond to their mechanical environment, ultimately modifying the valve's material properties [3–6]. Given this knowledge, we hypothesized that the altered mechanical stimulation of AVs in LVAD patients affects the AV's cells, the matrix composition those cells produce, and ultimately the valve's material properties. The altered AV material properties then could contribute to AR development long-term.

Accepted for publication Aug 7, 2017.

Address correspondence to Dr Stephens, 177 Fort Washington Ave, 5GN-435, Columbia University Medical Center, New York, NY 10033; email: ehs2140@cumc.columbia.edu.

The Supplemental Tables and Appendix can be viewed in the online version of this article [https://doi.org/10.1016/j.athoracsur.2017.08.030] on http://www.annalsthoracicsurgery.org.

0003-4975/$36.00
https://doi.org/10.1016/j.athoracsur.2017.08.030

Table 1. Demographics and Preoperative Clinical Status

Variable	LVAD (n = 16)	Non-LVAD (n = 6)	*p*
Demographics			
Age (years)	55.8 ± 8.0	51.8 ± 10.5	0.35
Male	15 (93.8%)	4 (66.7%)	0.17
NICM	9 (56.3%)	3 (50.0%)	1.00
Pretransplant echocardiogram			
AV			0.48
≤Mild AR	15 (93.8%)	5 (83.3%)	
≥Mild-Mod AR	1 (6.2%)	1 (16.7%)	
AV opens each beat	7 (43.8%)	NA	NA
MV			1.00
≤Mild MR	12 (75%)	5 (83.3%)	
≥Mild-Mod MR	4 (25%)	1 (16.7%)	
TV			1.00
≤Mild TR	9 (56.2%)	4 (66.7%)	
≥Mild-Mod TR	7 (43.8%)	2 (33.3%)	
LVEF (%)	15.0 ± 8.5	15.8 ± 4.9	0.82
Right ventricle			0.34
≤Mild-Mod Reduced	9 (56.3%)	2 (33.3%)	
≥Mod Reduced	3 (18.7%)	3 (50.0%)	
Severely Reduced	4 (25.0%)	1 (16.7%)	
LVEDD (cm)	5.53 ± 1.10	5.98 ± 1.06	0.40
Previous cardiac surgery			
MV repair/replacement	4 (25.0%)	1 (16.7%)	1.00
TV repair	3 (18.8%)	1 (16.7%)	1.00
CABG	2 (12.5%)	0 (0%)	1.00
Preoperative clinical status			
Infusions for heart failure management	3 (18.8%)	6 (100%)	0.001
Additional device support	1 (6.3%)	0 (0%)	1.00
AICD	15 (93.8%)	6 (100%)	1.00
CVA/ICH	4 (25.0%)	1 (16.7%)	1.00
Device complications[a]	12 (75.0%)	NA	NA
LVAD type			
Heartmate II	12 (75.0%)	NA	NA
Heartmate 3	3 (18.8%)	NA	NA
HVAD	1 (6.2%)		NA
Duration LVAD (days)	477 ± 410	NA	NA

[a] Device complications included gastrointestinal bleeding (n = 4), ventricular arrhythmias (n = 2), infection (n = 4), stroke (n = 1), right heart failure (n = 3), and thrombosis (n = 3).

AICD = automatic implantable cardioverter-defibrillator; AR = aortic regurgitation; AV = aortic valve; CABG = coronary artery bypass graft; cm = centimeter; CVA = cerebral vascular accident; ICH = intracranial hemorrhage; LVAD = left-ventricular assist device; LVEDD = left-ventricular end diastolic diameter; LVEF = left ventricular ejection fraction; Mod = moderate; MV = mitral valve; MR = mitral regurgitation; NA = not applicable; NICM = non-ischemic cardiomyopathy; Preop = preoperative; TV = tricuspid valve; TR = tricuspid regurgitation.

Although the clinical impact of AR in LVAD patients has been widely reported, analysis of the structural, mechanical, and compositional changes in the AV associated with the development of AR have previously largely been limited to hematoxylin and eosin staining and gross pathology [7–9]. Two recent studies, however, analyzed LVAD AVs in more detail: the first found increased smooth muscle alpha-actin in the ventricularis layer of AVs from LVAD patients [10] and the second found no difference in fibroblast proliferation and mucopolysaccharide content between LVAD and non-LVAD samples [11]. Further insight into the mechanisms contributing to the development of AR may lead to interventions to prevent this debilitating complication in our LVAD patients.

Table 2. Tensile Mechanical Properties

Variable	LVAD (n = 13)	Non-LVAD (n = 6)	*p*
Elastic modulus (MPa)			
Circumferential	1.8 ± 0.7	1.8 ± 0.6	0.58
Radial	1.5 ± 0.5	1.4 ± 0.5	0.82
Maximum stress (MPa)			
Circumferential	0.25 ± 0.13	0.29 ± 0.12	0.52
Radial	0.29 ± 0.10	0.35 ± 0.17	0.35
Maximum strain (MPa)			
Circumferential	0.35 ± 0.10	0.52 ± 0.18	**0.03**
Radial	0.57 ± 0.14	0.74 ± 0.22	**0.06**

Bolded *p* values indicate statistically significant difference between groups.

LVAD = left-ventricular assist device.

Patients and Methods

AVs from 16 consecutive LVAD and 6 non-LVAD patients from March 2015 through November 2016 were collected at the time of heart transplant at our institution, under a protocol approved by the institutional review board. Patients with infiltrative diseases were excluded, as were LVAD patients with a Park stitch AV closure. Standard demographic and clinical data were collected, including the degree of AV opening on last transthoracic echocardiogram prior to transplant. AV opening was graded as none, intermittent, or opening each beat.

Details of the mechanical testing methods are in the Supplemental Appendix. Briefly, a 10 mm × 10 mm specimen from the AV central portion of 13 LVAD and 6

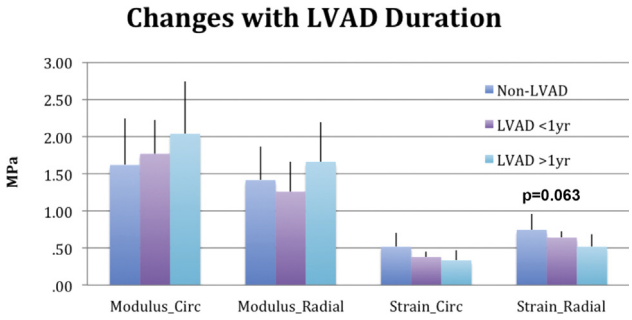

Fig 1. Mechanical property changes with increasing left-ventricular assist device (LVAD) duration. (Circ = circumferential.)