Aortic Valve Pathology in Patients Supported by Continuous-Flow Left Ventricular Assist Device

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Background: Continuous-flow left ventricular assist devices (CF-LVAD) may induce pathological changes to the aortic wall and aortic valve. We assessed histological changes in the relevant anatomic structures exposed to continuous flow over time and compared the histological results with clinical features in patients supported with CF-LVAD.

Methods and Results: A retrospective histological analysis was performed of 38 explanted hearts supported with CF-LVAD from patients who received heart transplantation between July 2003 and February 2014. Sections of formalin-fixed paraffin-embedded tissue showing the continuity of aortic wall and left-sided valves were examined histologically. Thickness of aorta, aortic root and aortic valve as well as 3 layers of the aortic cusps were measured individually on Elastica van Gieson-stained slides using specific software. Clinical parameters concerning aortic valve dysfunction were evaluated and validated against the histology. The aortic valve spongiosa and fibrosa layers showed no significant differences in thickness with regard to support duration or occurrence of aortic insufficiency. Longer CF-LVAD support duration correlated with a thinner aortic valve ventricularis layer (rs=-0.496).

Conclusions: Long-term CF-LVAD support appears to cause involution of the ventricularis layer of the aortic valve cusp, consistent with more pronounced degenerative change with longer LVAD exposure, which may be explained by continuous coaptation of the cusps. (Circ J 2016; 80: 1371–1377)

Key Words: Aortic valve regurgitation and pathology; Assisted circulation; Ventricular assist device

In the current era of improved clinical outcomes with ventricular assist device (VAD) therapy, the management and treatment of associated complications have come into focus. Alteration of flow dynamics in the ascending aorta produced by unphysiological continuous blood flow of a left VAD (LVAD) outflow graft anastomosed to the ascending aorta may induce structural changes in the aortic valve, sinus of Valsalva and aortic valve. De novo aortic insufficiency (AI) is one such complication. In the clinical setting, de novo AI of mild or higher grade has been reported to occur in 14.3–52% of patients at a median of 90–187 days after continuous-flow LVAD (CF-LVAD) implantation.1–3 Morbidity, mortality, and management of this complication have been frequently discussed but the underlying mechanisms are still poorly understood.

Commissural fusion of aortic valve has been observed frequently in patients with de novo AI after LVAD implantation,4 but no novel histological finding has been obtained except for anecdotal reports. The aim of this study was therefore to assess the histological changes in the anatomic structures exposed to continuous-flow stress produced by CF-LVAD over time. These findings were then evaluated in the context of clinical findings and analysis of echocardiographic data.

Methods

This study was approved by the institutional review board of Deutsches Herzzentrum Berlin and informed consent was obtained from each patient.

Medical records of all the patients supported with CF-LVAD and finally bridged to heart transplantation were retrospectively reviewed. Echocardiography was also reviewed. The AI grading system used during LVAD support was based on AI jet width, as follows: grade 0, none; 0.5, minimal; 1, mild; 1.5, mild-moderate; 2, moderate; 2.5, moderate-severe; and 3, severe. Status of the aortic valve opening was divided into 3 grades: normal (normal opening at every cardiac cycle); intermittent (approximately 1 opening motion in every 3 cardiac cycles); and absent (valve remains closed). Patients whose valve opening status varied with follow-up echocardiogram were assessed on the basis of serial changes of the echocardiographic findings and received a comprehensive judgment, mostly being classified as intermittent. Prevalence of de novo AI, clinical factors influencing the occurrence of de novo AI
and freedom from de novo AI ≥ grade 1 were assessed.

Histological samples from native hearts supported with an LVAD that were removed during heart transplantation were examined on microscopy. Sections of formalin-fixed paraffin-embedded tissue showing the continuity of aortic wall and left-sided valves were assessed by a single examiner under the supervision of a cardiovascular histopathologist. Left coronary cusp samples were taken through the midline perpendicular to the nadir of the sinus of Valsalva but avoiding the Arantius body because the nodule of Arantius contains a wide variation of characteristics even in the same patient. Aortic wall samples were taken as contiguous sections to the aortic valve samples. Outflow graft was anastomosed at the anterior surface of the ascending aortic wall in all patients studied, then, the aortic wall was sampled approximately 45–90° laterally to the site into which the jet from the outflow graft runs. The slides stained by conventional histology (hematoxylin and eosin) and a histochemical collagen stain (elastica van Gieson) were measured using dedicated software (NIS Elements version 4.10, Nikon Imaging, Japan). The following distances were measured (Figure 1): thickness of the intima and media of aortic root (sinus wall and tubular aorta) (proximal ascending aorta). The 3 individual layers of the aortic valve cusps (pars fibrosa; spongiosa; and ventricularis) and thickness index (thickness at free edge/thickness at basal part) were also measured to determine hypertrophic or atrophic change of each layer according to duration of LVAD support.

In our database 57 consecutive patients with CF-LVAD who were bridged to heart transplantation between July 2003 and February 2014 were identified. Nine of them aged <16 years at the time of LVAD implantation were excluded from the study. Patients with clinically significant aortic stenosis, history of aortic valve surgery or bicuspid aortic valve diagnosed before implantation of CF-LVAD were excluded from the study. The studied patients had no AI or less than minimal AI before CF-LVAD implantation. Accordingly, there was no patient who required a concomitant aortic valve procedure at the time of LVAD implantation. Additionally, materials considered to be unsuitable or insufficient for histological assessment were excluded. Eventually, 38 patients with 71 histological sections were involved in this study. As a clinical consideration in this cohort, 4 patients who underwent exchange of LVAD during the study period were included because the type of device (continuous-flow pump) was the same before and after the pump exchange procedure: Incor (Berlin Heart, Berlin, Germany) to Incor in 2; HeartWare HVAD (HVAD; HeartWare international, Framingham, MA, USA) to HVAD in 1 and Jarvik 2000 (Jarvik Heart, New York, NY, USA) to Incor in 1 patient.

Clinical parameters and transthoracic echocardiography were reviewed and compared with the histology to identify the pathological changes induced by continuous flow stress produced by CF-LVAD over time.

### De Novo AI and Valve Opening

AI of grade 1 developed in 11 patients (28.9%); grade 1.5 in 1 and grade 2 in 1 patient (2.6%) after LVAD implantation. There was no patient with AI ≥ grade 2.5. The 16 patients with mild or greater AI (21) had longer duration of CF-LVAD support (median, 640 days; range, 141–1,984 days) than the patients without AI (median, 262 days; range, 7–2,009 days; P=0.030; Table 1). There was an association between device type and development of AI ≥ grade 1. Patients with HM-II had higher prevalence of AI (72.7% at median support duration 570 days) than those with the other 2 devices (HVAD, 22.2% at median support duration 451 days; Incor, 44.4% at median support duration 840 days; P=0.028; Table 1).

Valve opening status was also associated with the development of AI. Two of 11 patients with normal motion of the aortic valve (18.2%) and 3 of 11 with intermittent opening of the aortic valve (27.3%) developed AI ≥ grade 1, whereas 11 of 16 patients with closed aortic valve (68.8%) developed AI ≥ grade 1 (P=0.016; Table 1). The cumulative incidence curve showed prevalence of de novo AI to be 11.1% at 1 year, 32.2% at 2 years, 72.9% at 3 years, 72.9% at 4 years and

### Clinical Data

The median age at LVAD implantation was 45 years (range, 17–61 years) and 92% of the patients were men (n=35). The median LVAD support duration was 434 days (range, 7–2,009 days). The etiology of heart failure was non-ischemic dilated cardiomyopathy in 27, ischemic cardiomyopathy in 8, congenital heart disease in 2 and hypertrophic obstructive cardiomyopathy in 1 patient. One patient with hypertrophic obstructive cardiomyopathy underwent concomitant septal myectomy and another 2 patients underwent concomitant tricuspid valvuloplasty at the time of LVAD implantation. HeartMate II (HM-II; Thoratec, Pleasanton, CA, USA), HVAD and Incor were used in 11, 18 and 9 patients for median support duration of 433 days (range, 81–1,397 days), 218 days (range, 7–1,035 days) and 811 days (range, 281–2,009 days), respectively (Table 1).

### Statistical Analysis

Statistical analysis was carried out using SPSS 22.00 (SPSS, Chicago, IL, USA). Categorical data, expressed as absolute and relative frequencies, were compared using the Fisher exact test or the Pearson chi-squared test, as appropriate. Due to the highly skewed distributions, continuous data are expressed as median and range unless otherwise noted, and were compared using the Wilcoxon rank sum test. Correlations were analyzed with Spearman’s r. P<0.05 was considered statistically significant.

### Results
Aortic Valve Histology

The basal part of the aortic valve cusp works as a hinge during the opening motion of the normal cardiac cycle. We assumed it to be theoretically less changeable in its thickness. Indeed, the thickness of basal aortic valve cusp had no specific correlation with duration of support (r_s=–0.198; Spearman’s rho), age (r_s=0.035; Spearman’s rho), prevalence of de novo AI (P=0.569) or history of hypertension (P=0.529).

Therefore, we used the data collected from the basal part as a control reference for the thickness at the free edge of each aortic cusp layer. To assess the degree of thickening of the free edge, measured data were indexed to the thickness of the basal part.

Overall, mean thickness of the whole cusp, fibrosa, spongiosa

Table 1. Patient Clinical Characteristic vs. De Novo AI After CF-LVAD Implantation

<table>
<thead>
<tr>
<th>Parameters</th>
<th>All patients (n=38)</th>
<th>De novo AI (≥1) (n=16)</th>
<th>No AI (0 or 0.5) (n=22)</th>
<th>P-value vs. AI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration (days)</td>
<td>434 (7–2,009)</td>
<td>640 (141–1,984)</td>
<td>262 (7–2,009)</td>
<td>0.030</td>
</tr>
<tr>
<td>Age (years)</td>
<td>45 (17–64)</td>
<td>42.5 (18–61)</td>
<td>46 (17–64)</td>
<td>0.700</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>Female</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>35 (92.1)</td>
<td>3 (7.9)</td>
<td></td>
<td>0.562</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>DCM</td>
<td>ICM</td>
<td>CHD</td>
<td>HCM</td>
</tr>
<tr>
<td></td>
<td>27 (71.1)</td>
<td>8 (21.1)</td>
<td>2 (5.3)</td>
<td>1 (2.6)</td>
</tr>
<tr>
<td></td>
<td>12 (75)</td>
<td>4 (25)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>15 (68.2)</td>
<td>4 (18.2)</td>
<td>2 (9.1)</td>
<td>1 (4.5)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>16 (42.1)</td>
<td>9 (56.3)</td>
<td>7 (31.8)</td>
<td>0.188</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>8 (21.1)</td>
<td>5 (31.3)</td>
<td>3 (13.6)</td>
<td>0.243</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>8 (21.1)</td>
<td>2 (12.5)</td>
<td>6 (27.3)</td>
<td>0.426</td>
</tr>
<tr>
<td>Obesity (BMI &gt;30)</td>
<td>10 (26.3)</td>
<td>4 (25)</td>
<td>6 (27.3)</td>
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<td>Smoking</td>
<td>14 (36.8)</td>
<td>5 (31.3)</td>
<td>9 (40.9)</td>
<td>0.735</td>
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<td>COPD</td>
<td>3 (7.9)</td>
<td>2 (12.5)</td>
<td>1 (4.5)</td>
<td>0.562</td>
</tr>
<tr>
<td>CRF (Cr &gt;1.6mg/dl)</td>
<td>10 (26.3)</td>
<td>4 (25)</td>
<td>6 (27.3)</td>
<td>1.000</td>
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<tr>
<td>Hyperuricemia</td>
<td>2 (5.3)</td>
<td>1 (6.3)</td>
<td>1 (4.5)</td>
<td>1.000</td>
</tr>
<tr>
<td>Type of VAD</td>
<td>HeartMate II</td>
<td>HeartWare</td>
<td>Incor</td>
<td></td>
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<tr>
<td></td>
<td>11 (29)</td>
<td>18 (47)</td>
<td>9 (24)</td>
<td>0.028</td>
</tr>
<tr>
<td></td>
<td>8 (50)</td>
<td>4 (25)</td>
<td>4 (25)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 (13.6)</td>
<td>14 (63.6)</td>
<td>5 (22.7)</td>
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<tr>
<td>Previous heart surgery</td>
<td>4 (11)</td>
<td>2 (12.5)</td>
<td>1 (4.5)</td>
<td>0.624</td>
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<td>Previous CABG</td>
<td>2 (5.3)</td>
<td>0 (0)</td>
<td>2 (9.1)</td>
<td>0.499</td>
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<td>Previous PCI</td>
<td>4 (11)</td>
<td>2 (12.5)</td>
<td>2 (9.1)</td>
<td>1.000</td>
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<tr>
<td>Pump exchange</td>
<td>4 (11)</td>
<td>3 (18.8)</td>
<td>1 (4.5)</td>
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<td>Cable dysfunction</td>
<td>2 (5.3)</td>
<td>1 (3.8)</td>
<td>1 (4.5)</td>
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<td>Thrombus</td>
<td>2 (5.3)</td>
<td>2 (12.5)</td>
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<td>Valve status</td>
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<td>Intermittent</td>
<td>Closed</td>
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</tr>
<tr>
<td></td>
<td>11 (29)</td>
<td>11 (29)</td>
<td>16 (42)</td>
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</tr>
<tr>
<td></td>
<td>2 (12.5)</td>
<td>3 (18.8)</td>
<td>11 (75)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9 (41)</td>
<td>8 (36.4)</td>
<td>5 (22.7)</td>
<td></td>
</tr>
<tr>
<td>Echocardiogram</td>
<td>LVEF (%)</td>
<td>15.8 (8–25)</td>
<td>19.9 (10–30)</td>
<td>0.961</td>
</tr>
<tr>
<td></td>
<td>73 (59–97)</td>
<td>70.5 (59–88)</td>
<td>74 (59–97)</td>
<td>0.474</td>
</tr>
</tbody>
</table>

Data given as median (range) or n (%). AI, aortic insufficiency; BMI, body mass index; CABG, coronary artery bypass grafting; CF-LVAD, continuous-flow left ventricular assist device; CHD, congenital heart disease; COPD, chronic obstructive pulmonary disease; Cr, creatinine; CRF, chronic renal failure; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; ICM, ischemic cardiomyopathy; LVAD, left ventricular assist device; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; MVP, mitral valve plasty; PCI, percutaneous coronary intervention; TVP, tricuspid valve plasty.

Outcome of Closed Valve

We studied the development of AI ≥grade 1 among the 16 patients whose aortic valve remained closed. Aortic valve was closed for 59 days (range, 14–981 days) after initiation of LVAD support. Subsequently, 10 patients developed AI 157 days (range, 2–586 days) after the date the valve was documented to be closed, but 1 patient developed AI before the valve was documented as closed and later the valve status was changed to closed valve. Five patients developed no AI, although their aortic valves remained closed during LVAD support until heart transplantation.

Histology

Histology

Histology

72.9% at 5 years after implantation of CF-LVAD. Fifty percent of the patients developed de novo AI at 2.2 years after implantation of CF-LVAD (Figure 2).
fibrosa thickness had no association with duration of LVAD support (Figure 3).

Patients with longer duration of LVAD support had a thinner ventricularis layer at the free edge of the aortic valve cusp (rS = −0.496; Spearman’s rho). The spongiosa thickness and fibrosa thickness had no association with duration of LVAD support (Figure 3).

Mucopolysaccharide accumulation (23.7%), fibroblast proliferation (7.9%) and nodular thickening (44.7%, due to either myxoid degeneration or collagen accumulation) at the free edge of the cusp were observed independently of LVAD support duration and occurrence of AI (Table 3). Thrombus formation at the cusp was observed in 1 patient but the patient had reported no signs of a thrombotic event clinically.
Aortic Valve Histology

Influence of Device Type on De Novo AI

Another distinct result compared with previous studies was the correlation between prevalence of de novo AI and the type of LVAD. There was lower incidence of AI in patients with HVAD (22.2% in 18 patients) than with HM-II (72.7% in 11 patients), although the number of patients studied was low. Clinical studies carried out in the USA, where HM-II has been used as the main device (between 94 and 100% of all studied CF-LVAD patients) with a longer observation period, reported that 52–64% of HM-II patients had developed significant AI.\(^1\)\(^2\)\(^6\)\(^7\)

Alternatively, a UK study reported the incidence of mild or greater AI to be 43.1% in HM-II vs. 65.7% in HVAD recipients,\(^3\) the opposite of the present findings. In contrast, Soleimani et al reported that 0/8 patients (0%) developed AI at a mean of 158 days with the HVAD vs. 6/55 (10.9%) with the HM-II at a mean of 314 days.\(^11\)

Higher flow pulsatility and afterload sensitivity of the centrifugal pumps helps to avoid the less frequent opening of the aortic valve experimentally.\(^12\)\(^13\)

Overall Prevalence of AI After LVAD Support

The present prevalence of AI is lower than reported, although 43% of the patients had mild or greater AI (≥1) at a median duration of 434 days of LVAD support. No patients had severer AI (grade 2.5 or 3) and the severest grade was moder-
periods of clinical observation and larger subject groups are warranted to support the present clinical results.

**Aortic Valve Histological Anatomy**

The aortic valve cusp is structurally subdivided into 3 distinct layers: fibrosa; ventricularis; and spongiosa.\(^{14,15}\) The fibrosa is located on the aortic side of the cusp and is composed of collagen sheets and large bundles arranged in a circumferential direction.\(^{14,15}\) The thinner ventricularis covers the ventricular surface of the cusp and has considerably more elastin than the fibrosa.\(^{16,17}\) Between the fibrosa and the ventricularis is the spongiosa, consisting of a large amount of glycosaminoglycans and a few loosely connected fibrous proteins.\(^{14,15,16}\) Macroscopically, the cusp is thinnest at the middle and becomes thicker at the free edge of each aortic valve cusp, especially at the nodule of Arantius.\(^{17}\) In accordance with autopsy-based observations, thickness of the cusp increases with age, while thickness of the cusp at the base remains relatively constant during different ages.\(^{19}\) Therefore, we used the basal part, which is reported to be unchangeable in thickness, as a control to evaluate the degree of thickening at the free edge of the aortic valve cusp.

**Aortic Valve Histological Changes**

In the present study, long-term LVAD support appeared to cause involution of the ventricularis layer of the aortic cusp. Retrograde flow stress produced by the ascending aortic graft causes the aortic valve to remain in the closed position for a longer time than without an LVAD. Under this condition, we hypothesized that the contacting surface of the cusp, the ventricularis layer, eventually loses its structure and function with atheromatous degeneration. The normal aortic valve receives its oxygen supply via a combination of intrinsic microcirculation and diffusion from the surface of the valve,\(^{20,21}\) but the diffusion component is blocked when the cusp remains closed, and microcirculation is the only way for it to receive the oxygen. Nevertheless, it was proven experimentally that only 30\% of the base region and only 3\% of the rest of the cusp region is vascularized.\(^{20}\) Longer coaptation time makes oxygen supply to the distal aortic cusp difficult. Any structural change in its anatomy induced in the present study seems to be due to ischemic processes.

**Aortic Wall Histology**

A prior clinical report described dilatation of the aortic wall influenced by CF-LVAD implantation,\(^{9}\) but histological studies have not always been in agreement with such clinical evidence. Depletion in the number of elastin fibers, increased elastic fiber fragmentation or degenerative change in smooth muscle cells in the medial layer under continuous flow stress have been frequently reported in histological studies.\(^{22–24}\) In contrast, thinner aortic wall was not a common finding in these studies. We found no changes in the thickness of the aortic intima or media relating to duration of CF-LVAD support, in agreement with some of them.\(^{23,25}\) A recent aortic wall histology study from Colorado, however, reported that heart failure patients supported with CF-LVAD had increased aortic wall thickness, when compared with heart failure patients without CF-LVAD and non-failing donors.\(^{24}\) Further study is needed to resolve the controversial result between the clinical and histological findings, and it is necessary to prove whether clinical signs of progressive aortic dilatation are related to aortic wall thinning or to other mechanisms such as derangement of cell structures in the medial layer.\(^{26}\)

**Study Limitations**

Histological sections showing continuity of the aortic wall and left-sided valves were longitudinally resected from the left coronary cusp of the aortic valve, therefore the present results may reflect a limited aspect of the aortic valve because the residual non-coronary cusp and right coronary cusp were not examined. As another limitation, the present study did not define the mechanism of commissural fusion of the aortic valve that has been frequently discussed. The relationship between the aortic valve commissural fusion and the histological changes in the fused aortic valve cusp is still unknown. As a third point, we studied changes of the aortic valve cusp after LVAD support, and therefore it is not clear whether the histological changes in the ventricularis layer of the aortic cusp occurred as a result of LVAD support or existed before the introduction of LVAD. Comparison in a controlled study with a longer study period is required to confirm the validity of the present results.

**Conclusions**

Long-term CF-LVAD support appears to cause involution of the ventricularis layer of the aortic valve, especially at the free edge of the cusp, which is consistent with more pronounced degenerative changes during long-term LVAD exposure. This may correlate with the continuous coaptation of the cusps.

**Acknowledgment**

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**Conflicts of Interest**

V.F. has received research grants from Biotronik and Boston Scientific, honoraria from Medtronic, Edwards, St. Jude Medical and Aesculap, and serves as a consultant for Phillips, HeartWare, Berlin Heart, Aesculap and Thoratec. The other authors declare no conflicts of interest.

**References**