Aus dem Institut für Thorax- und Herzchirurgie

Deutsches Herzzentrum Berlin

der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

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

zur Erlangung des akademischen Grades

Doctor medicinae (Dr. med.)

vorgelegt der Medizinischen Fakultät

Charité – Universitätsmedizin Berlin

von

Tomohiro Saito

aus Tokyo

Datum der Promotion: 16.06.2018

Table of Contents

List of Figures

List of Tables

List of Abbreviations

AI	-	aortic insufficiency
CF	-	continuous flow
LVAD	-	left ventricular assist device
VAD	-	ventricular assist device

0 Abstract

Introduction

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.

Methodology

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.

Results

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.

Abstract

Einleitung

Linksventrikuläre Unterstützungssysteme mit kontinuierlichem Fluss (contiuous-flow left ventricular assist device; CF-LVAD) können pathologische Veränderungen an der Aortenwand und der Aortenklappe hervorrufen. Wir untersuchten histologische Veränderungen an den relevanten anatomischen Strukturen, die über längere Zeit einem kontinuierlichen Fluss ausgesetzt waren und verglichen die histologischen Ergebnisse mit klinischen Befunden bei mit CF-LVAD unterstützten Patienten.

Methodik

38 explantierte Herzen von CF-LVAD-Patienten, die zwischen Juli 2003 und Februar 2014 Herztransplantation erhalten hatten, wurden retrospektiv histologisch analysiert. Abschnitte von formalinfixierten, in Paraffin eingebetteten Gewebeproben, welche die Aortenwand und der linksseitigen Herzklappen zusammenhängend darstellten, wurden hierfür histologisch untersucht. Die Schnitte wurden mittels Elastica-van-Gieson-Färbung aufbereitet und die Dicke der Aorta, der Aortenwurzel und der Aortenklappe sowie der drei Schichten der Aortenklappensegel unter Verwendung spezifischer Software gemessen. Klinische Parameter einer Aortenklappendysfunktion wurden ausgewertet und mit der Histologie verglichen.

Ergebnisse

Die spongiosa- und fibrosa-Schichten der Aortenklappensegel zeigten keine signifikanten Unterschiede in ihrer Dicke bezogen auf die Dauer Unterstützung oder das Auftreten von Aorteninsuffizienz. Längere CF-LVAD-Unterstützungszeiten korrelierten mit einer dünneren ventricularis-Schicht der Segel ($r_s = -0{,}496$).

Schlussfolgerung

Es konnte geziegt werden, dass eine langfristige CF-LVAD-Unterstützung eine Involution der ventricularis-Schicht der Segel der Aortenklappe, sowie mit länger andauernder LVAD-Exposition auch ausgeprägte degenerative Veränderungen verursachen kann. Dies kann durch eine kontinuierliche Adaptation der Segel erklärt werden.

1 Introduction

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 LVAD outflow graft anastomosed to the ascending aorta may induce structural changes in the aortic wall, sinus of Valsalva and aortic valve. De novo 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, [4–6] 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.

2 Methods

2.1 Clinical investigation

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.

2.2. Histological observation

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 degree laterally to the site into which the jet from the outflow graft runs.

2.3 Measurement method

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 the 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.

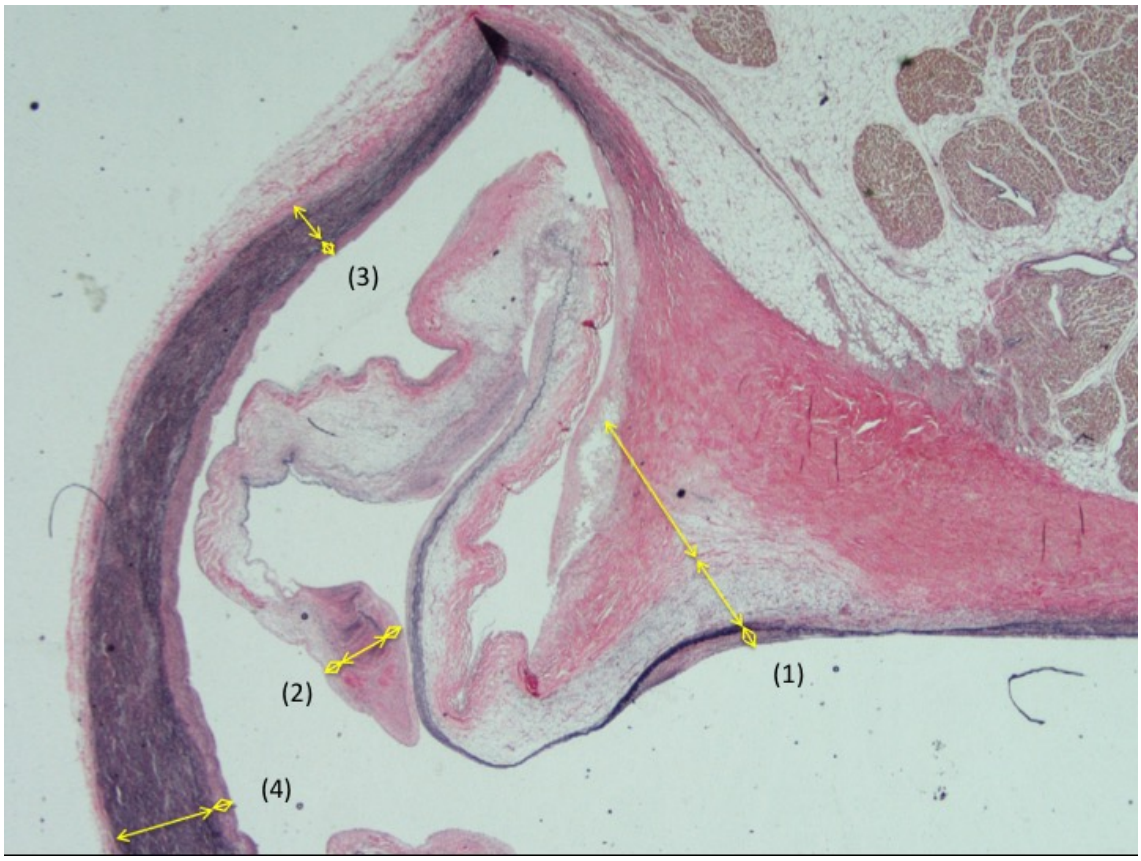

Figure 1. Histological elastica van Gieson stain showing the location of measurements.

(1) Individual 3 layers of aortic valve cusp at base. (2) Individual 3 layers of aortic valve cusp at free edge as distally as possible. (3) Thickness of intima and media of aortic root in the middle portion. (4) Intima and media thickness of tubular aorta.

2.4 Study population and exclusion criteria

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.

2.5 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 rho. $P<0.05$ was considered statistically significant.

3 Results

3.1 Clinical data

3.1.1 Overall indication

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 (HMII; 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).

3.1.2 Aortic valve function

AI of grade 1 developed in 11 patients (28.9%); grade 1.5 in 4 (10.5%); 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 (≥1) had longer duration of CF-LVAD support (median, 640 days; range, 141–1,984 days) than the patients without AI (median, 262 days; range, 7–2009 days; P=0.030; Table 1).

Table 1: Patient clinical characteristics vs. De novo AI after CF-LVAD implantation

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

Parameters	All patients (n=38)	De novo AI (≥1) (n=16)	No AI (0 or 0.5) (n=22)	P-value vs. AI
Duration (days)	434 (7–2,009)	640 (141–1,984)	262 (7–2,009)	0.030
Age (years)	45 (17–64)	42.5 (18–61)	46 (17–64)	0.700
Sex				0.562
Male	35 (92.1)	14 (87.5)	21 (95.5)	
Female	3 (7.9)	2 (12.5)	1 (4.5)	
Diagnosis				0.485
DCM	27 (71.1)	12 (75)	15 (68.2)	
ICM	8 (21.1)	4 (25)	4 (18.2)	
CHD	2 (5.3)	0 (0)	2 (9.1)	
HCM	1 (2.6)	0 (0)	1 (4.5)	
Hypertension	16 (42.1)	9 (56.3)	7 (31.8)	0.188
Hyperlipidemia	8 (21.1)	5 (31.3)	3 (13.6)	0.243
Diabetes mellitus	8 (21.1)	2 (12.5)	6 (27.3)	0.426
Obesity (BMI >30)	10 (26.3)	4 (25)	6 (27.3)	1.000
Smoking	14 (36.8)	5 (31.3)	9 (40.9)	0.735
COPD	3 (7.9)	2 (12.5)	1 (4.5)	0.562
CRF (Cr >1.6 mg/dl)	10 (26.3)	4 (25)	6 (27.3)	1.000
Hyperuricemia	2 (5.3)	1 (6.3)	1 (4.5)	1.000
Type of VAD				0.028
HeartMate II	11 (29)	8 (50)	3 (13.6)	
HeartWare	18 (47)	4 (25)	14 (63.6)	
Incor	9 (24)	4 (25)	5 (22.7)	
Previous heart surgery	4 (11)	1 (3.8)	3 (13.6)	0.624
Previous CABG	2 (5.3)	0 (0)	2 (9.1)	0.499
Previous PCI	4 (11)	2 (12.5)	2 (9.1)	1.000
Pump exchange	4 (11)	3 (18.8)	1 (4.5)	0.291
Cable dysfunction	2 (5.3)	1 (3.8)	1 (4.5)	
Thrombus	2 (5.3)	2 (12.5)	0 (0)	
Valve status				0.016
Normal	11 (29)	2 (12.5)	9 (41)	
Intermittent	11 (29)	3 (18.8)	8 (36.4)	
Closed	16 (42)	11 (75)	5 (22.7)	
Echocardiogram				
LVEF (%)	15 (8–30)	15.8 (8–25)	16.9 (10–30)	0.961
LVEDD (mm)	73 (59–97)	70.5 (59–88)	74 (59–97)	0.474

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.

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 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).

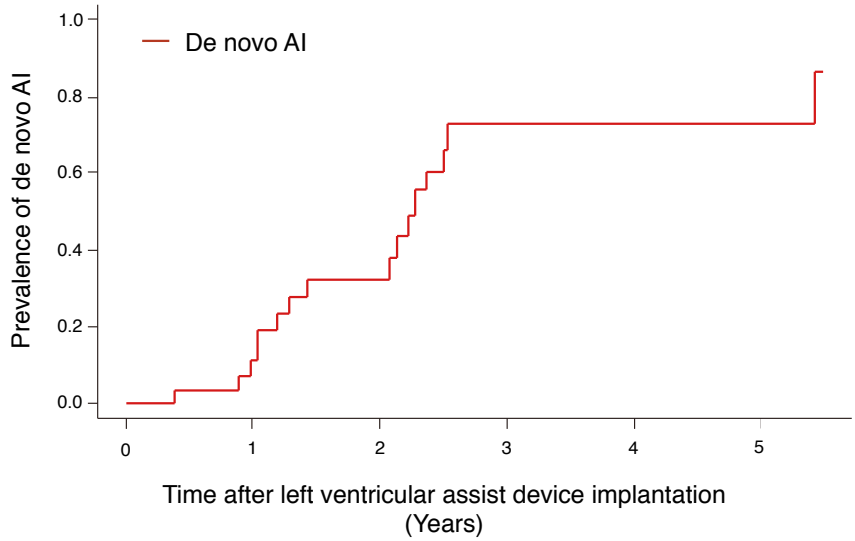

Figure 2. Cumulative incidence curve.

50% of the patients developed de novo aortic insufficiency (AI) ≥grade 1 at 2.2 years after implantation of continuous-flow left ventricular assist device (CF-LVAD). More than 75% of the patients developed de novo AI within 3 years after implantation of CF-LVAD.

3.1.3 Outcome of the patients with aortic valve dysfunction

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.

3.2 Histological results

3.2.1 Aortic valve

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 (rS=–0.198; Spearman's rho), age (rS=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. Thickness and index of each layer are listed in Table 2.

Table 2: Histology vs. De novo AI and hypertension.

Table 2. Histology vs. De Novo AI and Hypertension

Measured anatomy		Total (n=38)	De novo AI (+) (n=16, 42%)	De novo AI (–) (n=22, 58%)	P-value	Hypertension (+) (n=16, 42%)	Hypertension (–) (n=22, 58%)	P-value
Aortic valve								
Whole cusp	Thickness (μm)	553 (213–1,703)	586 (213–1,703)	531 (301–1,192)	0.510	668 (213–1,703)	497 (260–1,192)	0.045
Fibrosa	Index	0.44 (0.16–1.79)	0.44 (0.21–1.79)	0.46 (0.16–1.69)	0.804	0.46 (0.16–1.79)	0.43 (0.21–1.69)	0.781
	Thickness (μm)	256 (62–690)						
Spongiosa	Index	0.47 (0.06–2.16)	0.49 (0.06–2.16)	0.45 (0.13–0.87)	0.372	0.59 (0.06–0.98)	0.36 (0.13–2.16)	0.255
	Thickness (μm)	201 (49–1,067)						
Ventricularis	Index	0.56 (0.07–6.48)	0.51 (0.07–4.28)	0.56 (0.11–6.48)	0.781	0.77 (0.25–4.28)	0.49 (0.07–6.48)	0.069
	Thickness (μm)	89 (21–1,048)						
Aortic root								
Intima	Thickness (μm)	72 (38–342)	66 (40–157)	76 (38–342)	0.358	68 (54–109)	82 (38–342)	0.401
Media	Thickness (μm)	844 (270–2,344)	1,072 (309–2,092)	809 (270–2,344)	0.606	948 (270–2,092)	809 (300–2,344)	0.901
Ascending aorta								
Intima	Thickness (μm)	95 (43–139)	95 (59–309)	90 (43–1,391)	0.770	95 (59–363)	91 (43–1391)	0.984
Media	Thickness (μm)	1,347 (599–2,529)	1,421 (728–2,529)	1,269 (599–2,210)	0.929	1,404 (728–2,529)	1,269 (599–2,210)	0.292

Data given as median (range). AI, aortic insufficiency.

Overall, mean thickness of the whole cusp, fibrosa, spongiosa and ventricularis was 622±309 μm (median, 553 μm; range, 213–1,703 μm); 291±122 μm (median, 256 μm; range, 62–690 μm); 218±126 μm (median, 201 μm; range, 49–1,067 μm); and 133±183 μm (median, 89 μm; range, 121–1,048 μm), respectively. Ventricularis thickness index was found to have a weak correlation 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).

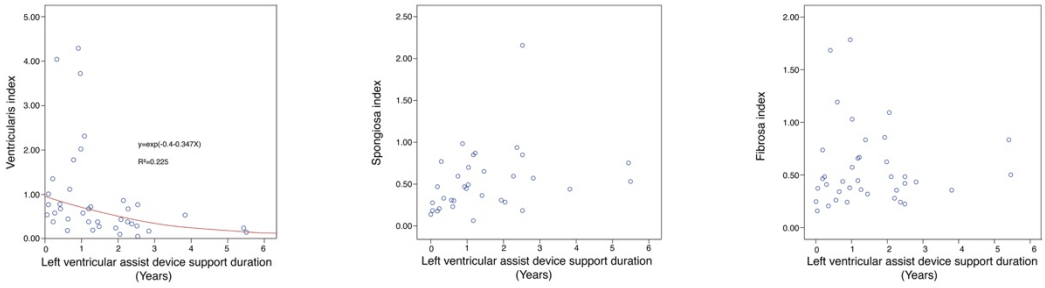

Figure 3. Relationship between the thickness index of each structural layer of the aortic valve cusp and the duration of left ventricular assist device (LVAD) support.

The ventricularis layer correlated with duration of continuous-flow LVAD (CF-LVAD) support. Patients supported for a longer duration had smaller ventricularis thickness. No significant correlation was found between spongiosa or fibrosa layer and duration of CF-LVAD support.

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.

Table 3: Prevalence of histological change in aortic valve cusp

Table 3. Prevalence of Histological Change in Aortic Valve Cusp							
	All patients (n=38)	De novo AI		P-value	Hypertension		P-value
		(+) (n=16, 42%)	(–) (n=22, 58%)		(+) (n=16, 42%)	(–) (n=22, 58%)	
Mucopolysaccharide	23.7 (9)	31.3 (5)	18.2 (4)	0.450	31.3 (5)	18.2 (4)	0.350
Fibroblast proliferation	7.9 (3)	12.5 (2)	4.5 (1)	0.562	18.8 (3)	0 (0)	0.034
Nodular thickening	44.7 (17)	56.3 (9)	36.4 (8)	0.324	50 (8)	40.9 (9)	0.578

Data given as % (n). AI, aortic insufficiency.

3.2.2 Sinus wall, tubular aorta and aortic root

The examined aortic wall, an extension of the left coronary cusp of the aortic valve, was obtained from the left posterolateral aspect of the aortic wall. Intima and media at the aortic root and ascending aortic wall showed no specific change in thickness with regard to duration of support, occurrence of de novo AI, or presence of hypertension (Table 2). Significant calcification or atherosclerotic changes were not remarkable in the samples.

4. Discussion

In the present histology study, the mechanism of de novo AI is likely to be multi-factorial. The present significant relationship between valve status and prevalence of de novo AI was similar to that in other reports.[2,6–10] Ten of 16 patients developed de novo AI on LVAD at a median of 157 days after the aortic valve was documented to be closed; in contrast, the remaining 6 patients had de novo AI irrespective of the valve opening status. We

speculate that the cause of AI in the first 10 patients may be related to structural changes of aortic valve induced by LVAD implantation, such as commissural fusion. In the latter 6 patients, however, AI may instead be related to existing incompetence of aortic valve before the LVAD implantation.

4.1 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 moderate (grade 2), in 1 patient (3%). Indeed, only a few patients have required later intervention for the aortic valve (the institutional protocol usually recommends aortic valve replacement) due to de novo AI on LVAD support, even as permanent support patients, since the start of the VAD treatment program. This may explain the lower prevalence of de novo AI in the present LVAD patients. In contrast, the present study group included younger patients than in other reports. This is presumably an alternative explanation for the lower occurrence rate of de novo AI because more of the present patients had intact aortic valve than in other reports.

4.2 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] Longer periods of clinical observation and larger subject groups are warranted to support the present clinical results.

4.3 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.[14,15,18] 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.

4.4 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 atrophic 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.

4.5 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]

4.6 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.

4.7 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.

5 Bibliography

1. Aggarwal A, Raghuvir R, Eryazici P, Macaluso G, Sharma P, Blair C, Tatooles AJ, Pappas PS, Bhat G. The development of aortic insufficiency in continuous-flow left ventricular assist device-supported patients. *Ann Thorac Surg* 2013;95:493–8.
2. Pak SW, Uriel N, Takayama H, Cappleman S, Song R, Colombo PC, Charles S, Mancini D, Gillam L, Naka Y, Jorde UP. Prevalence of de novo aortic insufficiency during long-term support with left ventricular assist devices. *J Heart Lung Transplant* 2010;29:1172-6.
3. Patil NP, Sabashnikov A, Mohite PN, Garcia D, Weymann A, Zych B, Bowles CT, Hards R, Hedger M, Popov AF, De Robertis F, Moza A, Bahrami T, Amrani M, Rahman-Haley S, Banner NR, Simon AR. De novo aortic regurgitation after continuous-flow left ventricular assist device implantation. *Ann Thorac Surg* 2014;98:850-7.
4. Rose A, Park S, Bank A, Miller L. Partial aortic valve fusion induced by left ventricular assist device. *Ann Thorac Surg* 2000; 70: 1270 – 1274.
5. Letsou GV, Connelly JH, Delgado RM 3rd, Myers TJ, Gregoric ID, Smart FW, Frazier OH. Is native aortic valve commissural fusions in patients with long-term left ventricular assist devices associated with clinically important aortic insufficiency? *J Heart Lung Transplant* 2006;25:395–9.
6. Mudd JO, Cuda JD, Halushka M, Soderlund KA, Conte JV, Russell SD. Fusion of aortic valve commissures in patients supported by a continuous axial flow ventricular assist device. *J Heart Lung Transplant* 2008;27:1269–74.

7. Cowger J, Pagani FD, Haft JW, Romano MA, Aaronson KD, Kolias TJ. The development of aortic insufficiency in left ventricular assist device-supported patients. *Circ Heart Fail* 2010;3:668–74.
8. Hatano M, Kinugawa K, Shiga T, Kato N, Endo M, Hisagi M, Nishimura T, Yao A, Hirata Y, Kyo S, Ono M, Nagai R. Less frequent opening of the aortic valve and a continuous flow pump are risk factors for postoperative onset of aortic insufficiency in patients with a left ventricular assist device. *Circ J* 2011;75:1147–55.
9. Imamura T, Kinugawa K, Fujino T, Inaba T, Maki H, Hatano M, Kinoshita O, Nawata K, Kyo S, Non M. Aortic insufficiency in patients with sustained left ventricular systolic dysfunction after axial flow assist device implantation. *Circ J* 2015;79:104–11.
10. Imamura T, Kinugawa K, Nitta D, Hatano M, Kinoshita O, Nawata K, Ono M. Advantage of pulsatility in left ventricular reverse remodeling and aortic insufficiency prevention during left ventricular assist device treatment. *Circ J* 2015;79:1994–9.
11. Soleimani B, Haouzi A, Manoskey A, Stephenson ER, El-Banayosy A, Pae WE. Development of aortic insufficiency in patients supported with continuous flow left ventricular assist devices. *ASAIO J* 2012;58:326–9.
12. Moazami N, Fukamachi K, Kobayashi M, Smedira NG, Hoercher KJ, Massiello A, Lee S, Horvath DJ, Starling RC. Axial and centrifugal continuous-flow rotary pumps: A translation from pump mechanics to clinical practice. *J Heart Lung Transplant* 2013;32:1–11.
13. Stanfiled JR, Selzmann CH. In vitro pulsatility analysis of axial-flow and centrifugal-flow left ventricular assist devices. *J Biomech Eng* 2013;135:34505.

14. Billingham ME. Normal heart. *In*: Sternberg SS, editor. Histology for pathologists, 2nd ed. Philadelphia: Lippincott-Raven, 1997;753–4.
15. Butcher JT, Mahler GJ, Hockaday LA. Aortic valve disease and treatment: The need for naturally engineered solutions. *Adv Drug Deliv Rev* 2011;63:242–68.
16. Vesely I. The role of elastin in aortic valve mechanics. *J Biomech* 1998;31:115-23.
17. Filion RJ, Ellis CG. A finite difference model of O2 transport in aortic valve cusps: Importance of intrinsic microcirculation. *Am J Physiol Heart Circ Physiol* 2003;285: H2099–104.
18. Christie GW. Anatomy of aortic heart valve leaflets: The influence of glutaraldehyde fixation on function. *Eur J Cardiothorac Surg* 1992;6(Suppl 1):S25–32, discussion S33.
19. Sahasakul Y, Edwards WD, Naessens JM, Tajik AJ. Age-related changes in aortic and mitral valve thickness: Implications for two dimensional echocardiography based on an autopsy study of 200 normal human hearts. *Am J Cardiol* 1998;62:424–30.
20. Weind KL, Ellis CG, Boughner DR. Aortic valve cusp vessel density: Relationship with tissue thickness. *J Thorac Cardiovasc Surg* 2002;123:333–40.
21. Weind KL, Bouhhner DR, Rigutto L, Ellis CG. Oxygen diffusion and consumption of aortic valve cusps. *Am J Physiol Heart Circ Physiol* 2001;281:H2604–11.
22. Westaby S, Bertoni GB, Clelland C, Nishinaka T, Frazier OH. Circulatory support with attenuated pulse alters human aortic wall morphology. *J Thorac Cardiovasc Surg* 2007;133:575–6.

23. Segura AM, Gregoric I, Radovancevic R, Demirozu ZT, Buja LM, Frazier OH. Morphologic changes in the aortic wall media after support with a continuous-flow left ventricular assist device. *J Heart Lung Transplant* 2013;32:1096–100.
24. Ambardekar AV, Hunter KS, Babu AN, Tuder RM, Dodson RB, Lindenfeld J. Changes in aortic wall structure, composition, and stiffness with continuous-flow left ventricular assist devices. *Circ Heart Fail* 2015;8:944–52.
25. Potapov EV, Dranishnikov N, Morawietz L, Stepanenko A, Rezai S, Blechschmidt C, Lehmkuhl HB, Weng Y, Pasic M, Hübler M, Hetzer R, Krabatsch T. Arterial wall histology in chronic pulsatile-flow and continuous-flow device circulatory support. *J Heart Lung Transplant* 2012;31:1171–6.
26. Lee M, Akashi H, Kato TS, Takayama H, Wu C, Xu K, Collado E, Weber MP, Kennel PJ, Brunjes DL, Ji R, Naka Y, George I, Mancini D, Farr M, Schulze PC. Vascular inflammation and abnormal aortic histomorphometry in patients after pulsatile- and continuous-flow left ventricular assist device placement. *J Heart Lung Transplant* 2016;35:1085-91.

6 Affidavit

I, Tomohiro Saito, certify under penalty of perjury by my own signature that I have submitted the thesis on the topic “Aortic Valve Pathology in Patients Supported by Continuous-Flow Left Ventricular Assist Device.“ I wrote this thesis independently and without assistance from third parties, I used no other aids than the listed sources and resources.

All points based literally or in spirit on publications or presentations of other authors are, as such, in proper citations (see "uniform requirements for manuscripts (URM)" the ICMJE www.icmje.org) indicated. The section on methodology (in particular practical work, laboratory requirements, statistical processing) and results (in particular images, graphics and tables) corresponds to the URM (s.o) and are answered by me. My contribution in the selected publication for this dissertation corresponds to those that are specified in the following joint declaration with the responsible person and supervisor.

The importance of this affidavit and the criminal consequences of a false affidavit (section 156,161 of the Criminal Code) are known to me and I understand the rights and responsibilities stated therein.

_____

Date Signature

Detailed Declaration of Contribution

Tomohiro Saito had the following share in the following publication:

Saito T, Wassilew K, Gorodetski B, Stein J, Falk V, Krabatsch T, Potapov E.
Aortic Valve Pathology in Patients Supported by Continuous-Flow Left Ventricular Assist Device. Circulation Journal. May 2016.

Contribution in detail:

writing of whole part of the article, clinical data collection from the patient chart, histological measurement of the resected slide sections, analysis and evaluation of the statistical data, submission of the article to the journal.

Signature, date and stamp of the supervising University teacher

_____

Signature of the doctoral candidate

_____

7 Curriculum Vitae

Mein Lebenslauf ist in der elektronischen Version der Dissertation aus datenschutzrechtlichen Gründen nicht enthalten.

8 Acknowledgements

Firstly, I would like to express my sincere gratitude and appreciation to my mentor Dr. Potapov for the continuous support of my M.D. Thesis study. Your largess and open-minded attitude to me was really helpful to keep my motivation on my study. It was really proud days that I could complete this clinico-pathological study under you, one of the most famous cardiac surgeon in the field of assist device all of the world.

My sincere thanks also goes to Dr. Wassilew who has supervised my histological observation. Although of my poor research experience, you always advised me so kindly with unstinting support. My study has never accomplished without your support. I really understood the importance of the research mind even in the daily life of clinical works.

I really thank Anne Gale, Editor in the Life Sciences of the Deutsches Herzzentrum Berlin for editorial assistance. The language, not only German but also English, was a tough work for me, but you always guided me clearly and taught me the importance of scientific concept should be included in the study.

Prof. Hetzer, who is, needless to say, a famous worldwide cardiac surgeon, gave me a great chance and the opportunity to struggle this novel clinico-pathological investigation. My investigation could not begin and not complete except the existence of you. You are my mentor in Germany and established the base of my life as a physician as well as a researcher.